Lifestyle and 25-hydroxyvitamin D, and its associations with cognitive function among Icelandic older adults: AGES-Reykjavik study

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Lífsstíll, D-vítamín búskapur og tengsl við vitræna getu á meðal aldraðra: Öldrunarrannsókn Hjartaverndar

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Ágrip

Markmið: Markmið þessarar doktorsrannsóknar var að rannsaka áhrif lífstílsþátta á þróun vitrænnar getu og áhrif á heilabilun á meðal aldraðra. Sérstök áhersla var lögð á að rannsaka áhrif D-vítamíns (25-hydroxy- D vítamín (25OHD)), hreyfingar og líkamsþyngdarstuðuls á vitræna getu.

Í grein I voru áhrif D-vítamíns á þróun vitrænnar getu á meðal aldraðra rannsökuð og áhrif hreyfingar skoðuð sérstaklega í þessu samhengi.

Í grein II var gerð rannsókn á lífstílsþáttum einstaklinga sem höfðu greinst með heilabilun og væga vitræna skerðingu (e. mild cognitive impairment), og þeir bornir saman við einstaklinga sem ekki höfðu greinst með vitrænar breytingar. Þá voru tengsl lífstílsþátta við styrk 25OHD rannsökuð sérstaklega innan þessara hópa.

Í grein III voru breytingar á líkamsþyngd, aldraðra án skertrar vitrænnar getu, rannsakaðar með langtíma eftirfylgni og möguleg áhrif á vitræna getu metin. Jafnframt, var rannsakað hvort breytingar á líkamsþyngd tengdust þróun vægrar vitrænnar skerðingar (e. mild cognitive impairment) eða þróun heilabilunar.

Aðferðir: Grein I lýsti tengslum á milli styrks 25OHD í blóði og þróunar vitrænnar getu með þversniði á meðal 4304 þátttakenda, 66-96 ára, sem voru án heilabilunar. Styrkur D- vítamíns í blóði var mældur sem 25-hydroxy D vítamín (25OHD), gildum var skipt upp á hefðbundinn hátt: <30nmól/L (8%), 30-50 nmól/L (25%) og hæðsta gildi, jafnframt viðmiðunargildi, var ≥ 50 nmól/L (67%). Vitræn geta var mæld út frá taugasálfræðilegum prófum sem skiptust niður í; hraði úrvinnslu (e. speed of processing), minni (e. memory function) og stýring (e. executive function). Þessar þrjár útkomu mælingar voru skilgreindar með háu og lágu skori. Lógitísk aðhvarfsgreining var notuð við útreikninga á líkindahlutfalli þar sem stjórnað var fyrir áhrifum truflandi þátta í þremur skrefum.

Grein II samanstóð af 5162 þátttakendum og var meðalaldur 77 ár, spönn 66-96. Lífstílsþáttum einstaklinga með heilabilun (fjöldi=307), væga vitræna skerðingu (fjöldi=492) og einstaklinga án vitrænnar skerðingar (fjöldi=4363), var lýst með lýsandi tölfræði, leiðrétt fyrir aldri. Þá var rannsakaður styrkur 25OHD í blóði meðal þessara þriggja hópa, með leiðréttri línulegri aðhvarfsgreiningu. Útreikningum var að lokum lagskipt eftir stöðu vitrænnar getu (heilabilun, væg

vitræn skerðing, engin vitræn skerðing) og línulegri aðhvarfsgreiningu beitt til að reikna út tengsl á milli lífstílsþátta og 25OHD með stigvaxandi leiðréttingu á truflandi þáttum í þremur skrefum.

Grein III var langtímarannsókn með 2620 þátttakendum, 66-96 ára, þar sem þátttakendum var fylgt eftir að meðaltali í 5,2 ár. Breytingum á líkamsþyngd var skipt upp í þrjá hópa eða samkvæmt því hvort að einstaklingar hefðu; [1)] misst þyngd, [2)] bætt við þyngd sína eða [3)] staðið í stað (viðmiðunar hópur). Vitræn geta var metin út frá hraða úrvinnslu (e. speed of processing), minni (e. memory function) og stýringu (e. executive function). Notast var við línulega aðhvarfsgreiningu til að reikna tengsl á milli breytinga á líkamsþyngd og breytinga á vitrænni getu og áhrifa á þróun vægrar vitrænnar skerðingar og heilabilunar.

Niðurstöður: Vísindagrein I: Jákvætt samband reyndist á milli 25OHD og vitrænnar getu. Tekið var sérstakt tillit til hreyfingar í þessu sambandi þar sem hreyfing reyndist ekki hafa afgerandi áhrif á sambandið. Til samanburðar við þátttakendur sem höfðu há D- vítamín gildi (> 50 nmól/L), þá höfðu þátttakendur sem reyndust með skort á D-vítamíni (< 30 nmól/L) minnkaðar líkur á háu skori á hraða (OR: 0,74, CI: 0,57-0,97), minni (OR: 0,61; CI: 0,47-0,79) og stýringu (OR: 0,76, CI: 0,57-1,0).

Vísindagrein II: Gildi 25OHD voru marktækt lægri á meðal eldri einstaklinga sem greinst höfðu með heilabilun (53,8 ± 19,6 nmól/L) og væga vitræna skerðingu (55,8 ± 19,0 nmól/L) til samanburðar við þá sem ekki höfðu vitræna skerðingu (57,6 ± 17,7 nmól/L). Samkvæmt niðurstöðum línulegrar aðhvarfsgreiningar hafði neysla á lýsi (7,1 - 9,2 nmól/L, $P < 0,001$) og inntaka fjölvítamíns (4,4-11,5 nmól/L, $P < 0,001$) marktækt aukandi áhrif á styrk 25OHD, þvert á hópa.

Aðrir mældir lífstílsþættir sýndu fram á marktæka fylgni við styrk 25OHD á meðal þeirra sem sýndu fram á eðlilega vitræna getu, en ekki á meðal þeirra sem höfðu greinst með væga vitræna skerðingu eða heilabilun. Þannig var hreyfing ≥3klst/viku (2,82 nmól/L, $P < 0,001$), líkamsþyngdarstuðull <30kg/m^2 (5,2 nmól/L, $P < 0,001$), reykleysi (4,8 nmól/L, $P < 0,001$), áfengisneysla (2,7 nmól/L, $P < 0,001$) og neysla á feitum fisk ≥3x/viku (2,6 nmól/L, $P < 0,001$) eingöngu tengt hærri styrk 25OHD á meðal einstaklinga með eðlilega vitræna getu.

Vísindagrein III: Á meðal þátttakenda, með eðlilega vitræna getu við upphaf rannsóknar, höfðu 843 (32,2%, -6,7 ± 3,8 kg) misst þyngd, 505 (19,3%, 5,7 ± 2,9 kg) voru með þyngdaraukningu og 1272 (48,5%, -0,1 ± 1,5 kg) voru stöðugir

í þyngd. Þátttakendur sem misstu þyngd voru marktækt líklegri til að hnigna í minni (β: -0,098, P < 0,001) og hraða (β: -0,092, P < 0,001) til samanburðar við þá sem voru stöðugir í þyngd. Breytingar á þyngd tengdust ekki marktækt stýringu. Þyngdarskerðing tengdist aukinni áhættu á vægri vitrænni skerðingu, á hinn bóginn þá tengdist þyngdaraukning aukinni áhættu á heilabilun þegar borið var saman við þá sem reyndust stöðugir í þyngd.

Ályktanir: Samkvæmt niðurstöðum þessarar rannsóknar er um þriðjungur aldraðra einstaklinga, sem býr sjálfstætt, með ónógt magn D-vítamíns í blóði (< 50 nmól/L). Niðurstöður gefa til kynna að einstaklingar sem eru án vitrænnar skerðingar og eru með D vítamíni gildi undir 30 nmól/L séu líklegri til að vera í lægra þrepi vitrænnar getu sé tekið mið af þeim einstaklingum sem eru yfir 50 nmól/L.

Niðurstöður benda til þess að einstaklingar sem eru með heilabilun séu með lægri D-vítamíngildi samanborið við einstaklinga með eðlilega vitræna getu. Svo virðist sem einstaklingar með heilabilun treysti í meira mæli á inntöku fæðubótaefna heldur en samanburðarhópur. Hreyfing var í lágmarki á meðal einstaklinga með heilabilun og væga vitræna skerðingu, þá reyndist hreyfing ekki hafa marktækt samband við D-vítamín í þessum tveimur hópum þar sem breytingar á vitrænni getu höfðu átt sér stað. Þrátt fyrir að einstaklingar með heilabilun og væga vitræna skerðingu hefðu óhagstæðari lífstíl heldur en einstaklingar án vitrænnar skerðingar þá skýrði munur á lífstíl ekki þann mun sem reyndist á D-vítamín gildum hópanna.

Niðurstöðu sýndu að einstaklingar sem urðu fyrir þyngdartapi höfðu aukna áhættu á hnignandi vitrænni getu. Niðurstöður sýndu jafnframt að einstaklingar sem bættu við þyngd sína höfðu aukna áhættu á að greinast með heilabilun. Breytingar á líkamsþyngd á meðal aldraðra gætu því verið mikilvægur orsakaþáttur í þróun vitrænnar getu. Almennt benda niðurstöður til þess að lífstíll hafi áhrif á vitræna getu eldri aldurshópa.

Lykilorð:

D- vítamínbúskapur, líkamsþyngdarstuðull, hreyfing, vitræna geta, væg vitræn skerðing, heilabilun.

Abstract

Aims: The aim of this thesis was to evaluate lifestyle factors and the associations with cognitive function and the development of dementia among older adults with special considerations for vitamin D, physical activity and body mass index. Additionally, to explore the characteristics of people with dementia and mild cognitive impairment .

Paper I: To investigate the association between 25-hydroxyvitamin D (25OHD) and cognitive function with particular consideration of physical activity in Icelandic older adults.

Paper II: To investigate lifestyle and 25-hydroxyvitamin D levels (25OHD) in old adults with dementia, MCI or normal cognitive status (NCS).

Paper III: To investigate the longitudinal associations between changes in body weight and declines in cognitive function and risk of MCI/dementia among community-dwelling old adults, with normal cognitive function at baseline.

Methods: Paper I was a cross-sectional study. Participants were old adults aged 65-96. The final analytical sample included 4304 non-demented participants. Serum 25OHD was categorized into deficient (≤ 30 nmol/L, 8%), insufficient (31-49 nmol/L, 25%) and normal-high levels (>50 nmol/L, 67%). Cognitive function assessments included measurements of memory function (MF), speed of processing (SP) and executive function (EF) all categorized as low and high (divided by 50th percentile). Multivariate logistic regression analysis was used to calculate the odds ratio (OR) for having a high cognitive function.

Paper II was a cross-sectional study, comprising 5162 subjects (65-96 years) who were stratified by cognitive status, i.e., dementia (n = 307), MCI (n = 492) and NCS (n = 4363). Lifestyle variables were assessed (physical activity, body mass index, cod liver oil consumption, supplements, smoking, alcohol and fatty fish consumption) and 25OHD (used as a continuous variable) was measured. The associations between lifestyle and 25OHD were calculated using linear models correcting for potential confounders.

Paper III was a longitudinal study, comprising a cohort of 2620 older adults, (65-96 years). Cognitive function outcomes included the speed of processing (SP), executive function (EF) and memory function (MF). Longitudinal changes

in body weight were classified into three groups; weight loss (WL), weight gain (WG) and stable weight (SW). The associations between weight changes and declines in cognitive function and risk of mild cognitive impairment/dementia were examined with multiple logistic regression models adjusting for confounding factors.

Results: In paper I serum 25OHD was positively associated with cognitive function. Adjustment for physical activity and other potential confounders diminished this association only partially. Compared to participants with normal-high levels of 25OHD, those with deficient levels had decreased odds for high SP (OR: 0.74, CI: 0.57-0.97), high MF (OR: 0.61; CI: 0.47- 0.79) and high EF (OR: 0.76, CI: 0.57-1.0).

In Paper II serum 25OHD concentrations were significantly lower in older people with dementia (53.8 ± 19.6 nmol/L) and in MCI (55.8 ± 19.0 nmol/L) than in NCS participants (57.6 ± 17.7 nmol/L). According to linear models' cod liver oil (7.1 - 9.2 nmol/L, $P < 0.001$) and dietary supplements (4.4-11.5 nmol/L, $P < 0.001$) were associated with higher 25OHD in all three groups. However, physical activity ≥3h/week (2.82 nmol/L, $P < 0.001$), body mass index < 30 kg/m^2 (5.2 nmol/L, $P < 0.001$), non-smoking (4. nmol/L, $P < 0.001$), alcohol consumption (2.7 nmol/L, $P < 0.001$) and fatty fish consumption ≥3x/week (2.6 nmol/L, $P < 0.001$) were related to higher 25OHD in NCS only but not in participants with dementia or MCI.

In Paper III the mean follow-up time was 5.2 years, 843 participants (32.2%) lost weight (-6.7 ± 3.8 kg), 505 (19.3%) gained weight (5.7 ± 2.9 kg) and 1272 (48.5%) were weight stable (-0.1 ± 1.5 kg). Participants who experienced WL were significantly more likely to have declined in MF (β: -0.098, P-value < 0.001) and SP (β: -0.092, P-value < 0.001) compared to the SW group. Weight changes were not associated with EF. Weight loss was associated with a higher risk of MCI, while WG was associated with higher dementia risk when compared to SW.

Conclusions: According to the results of this study, lifestyle is associated with cognitive function among older adults. Around a third of the participants who were free from dementia, had either deficient or insufficient levels of vitamin D and, those who were deficient were more likely to have a lower cognitive function, in all three domains, as compared to normal vitamin D levels.

Older people living in the community in Iceland with dementia, have lower 25OHD compared to healthy individuals, although the majority of them still have vitamin D levels within the normal range. Yet, older people with dementia rely

more on vitamin D supplements than their healthy counterparts. Physical activity reported among participants with dementia and MCI is low and is not associated with 25OHD. Although participants with dementia had a poorer lifestyle than healthy participants, differences in lifestyle did not fully explain the observed lower levels of 25OHD in the dementia group.

Individuals who lost weight had a higher risk of declining cognitive function, while separated analysis showed that weight gain might contribute to the risk of developing dementia. Significant BW changes in older adulthood may, independently, indicate impending changes in cognitive function. When preventing cognitive decline and dementia among older adults, public health care systems should generally consider the lifestyle approach.

Keywords: 25-hydroxyvitamin D (25OHD), body mass index, physical activity, cognitive function, mild cognitive impairment, dementia.

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Contents

List of abbreviations

AGESII-Reykjavik study = Age, Gene/Environment Susceptibility Reykjavik Study, second phase

AGES-Reykjavik study = Age, Gene/Environment Susceptibility Reykjavik Study

25OHD = 25-hydroxyvitamin D

EF = executive function

GLM = general linear model

MCI = mild cognitive impairment

MF= memory function

MMSE = Mini-Mental State Examination

OR = Odds ratio

β = βeta coefficient

CI = Confidence interval

SD = standard deviation

SP = speed of processing

RCT = randomized clinical trial

WHO = World Health Organization

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List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I. **Hrafnhildur Eymundsdottir,** Milan Chang, Ólöf Guðný Geirsdottir, Pálmi V Jónsson, Vilmundur Gudnason, Lenor Launer, María K Jónsdottir, Alfons Ramel. Serum 25-hydroxy vitamin D, physical activity and cognitive function among older adults. Published in the Journal of aging research and clinical practice.

II. **Hrafnhildur Eymundsdottir,** Milan Chang, Ólöf Guðný Geirsdottir, Lárus S. Guðmundsson, Pálmi V Jónsson, Vilmundur Gudnason, Lenor Launer, María K Jónsdottir, Alfons Ramel. Lifestyle and 25-hydroxy-vitamin D among community-dwelling old adults with dementia, mild cognitive impairment or normal cognitive function. Published in Aging clinical and experimental research

III. **Hrafnhildur Eymundsdottir,** Alfons Ramel, Ólöf Guðný Geirsdottir, Lárus S. Guðmundsson, Pálmi V Jónsson, Vilmundur Gudnason, Lenor Launer, María K Jónsdottir, Milan Chang. Bodyweight changes and longitudinal associations with cognitive decline among community-dwelling old adults. Submitted to Age and Aging

Declaration of contribution

Drafting of manuscripts: Hrafnhildur Eymundsdóttir drafted the manuscripts and papers already published. *Statistical analyses* were in the hands of Hrafnhildur Eymundsdóttir, Alfons Ramel, and Milan Chang (Gudjonsson). *Data collection* and preparation: Hrafnhildur Eymundsdóttir, Milan Chang (Gudjonsson), Alfons Ramel, Ólöf Guðný Geirsdóttir, Pálmi V. Jónsson, Lárus S. Guðmundsson, and Þórhallur I. Halldórsson.

María K. Jónsdóttir contributed to the cognitive function measurements from raw data to more finalized domains of cognitive function.

All authors contributed to the interpretation of the results, read and commented on the manuscripts and approved the final versions.

1 Introduction

Aging is a normal physiological process but can be associated with some degrees of decrease in cognitive function. Cognitive decline is considered abnormal when it is greater than among age-matched controls and is associated with an inability to perform activities of daily living, then the decline is referred to as dementia [1]. Dementia is not a normal part of aging, it is a heterogeneous neurodegenerative disorder, commonly described as a syndrome, rather than a specific disease [2], usually of a chronic or progressive nature. Mild cognitive impairment (MCI) is a less progressive state of cognitive decline. MCI is associated with functional independence, but the cognitive decline is greater than age-matched control. Around one-half of old adults with MCI progresses to dementia within five years, the other half remains stable or even returns to a normal state over time [4]. MCI can, therefore, be considered as a risk state for dementia [5].

Dementia is a national and global health crisis. The number of people living with dementia worldwide is currently estimated at 50 million and will almost triple by 2050 [6-8]. Studies show that by delaying the onset of dementia symptoms, preventive measures have the potential to reduce the prevalence of dementia dramatically in the coming decades [9]. Major risk factors for developing dementia include advancing age and genetic profile. However, delayed onset of symptoms may be achieved through interventions focusing on physical activity and nutrition, which may influence modifiable risk factors like systemic vascular disease [10, 11]. While medical treatment has been shown to slow down the progression of dementia, the clinical focus has been shifting towards detection and prevention [10]. Therefore, disseminating information on modifiable risk factors at the early stages of dementia for health care providers would help to adopt effective dementia prevention and care strategies at the community level.

The overall aim of the current study is to investigate the association between lifestyle factors, nutrition, and the risk of developing dementia. The study examines the relationship between serum vitamin D levels, physical activity, body mass index, and cognitive function. The study further examines lifestyle factors among individuals who have been diagnosed with dementia or MCI with cognitively intact participants. The study is based on cross-sectional and longitudinal data from the Age, Gene/Environment Susceptibility (AGES) - Reykjavik study.

1.1 Mild cognitive impairment and dementia symptomology

Early clinical diagnosis of dementia is important and much effort in the field of aging has been devoted to this early diagnosis of cognitive decline with multiple definitions. The aim of such definitions is to capture the intermediate stage between healthy aging and dementia. The most successful definition has been the term mild cognitive impairment, introduced to the clinical field more than 30 years ago [12, 13]. The first clinical criteria for MCI (1999) was narrow and focused mainly on deficits in memory domain. When these first criteria were investigated, it became clear that broader conceptualizations was necessary [14] which ultimately led to definition including impairments in other cognitive domains.

According to criteria from the National Institute on Aging - Alzheimer's Associations workgroup, four factors need to be involved [15]. First, there should be evidence of concerns in changes in cognition either from the patient, from a caregiver or from a professional health care provider. Second, objective impairment in one or more cognitive domains that is greater than would be expected for the patients age and educational background. Third, independence in functional abilities, such as paying bills, preparing a meal or shopping, should be preserved, even though minimal assistance is allowed. Fourth, the cognitive changes observed should not pass the threshold of dementia and therefor be sufficiently mild and without significant impairment in social and occupational functioning [15].

Complaints about poor memory are the most common reasons for referral to a memory clinic [16]. If a medical history and mental examinations do not provide a clear diagnosis of cognitive impairment, neuropsychological testing should be performed.

The National Institute on Aging - Alzheimer's Associations workgroup further proposed a criteria for the diagnosis of all-cause dementia. According to the workgroup, dementia is diagnosed when there are cognitive and behavioral symptoms that interfere with activities of daily living.

When cognitive and behavioral symptoms can not be explained by the presence of delirium or major psychiatric disorders neuropsychological testing should be performed. The neuropsychological testing should confirm impairment in at least two domains of cognitive function. The examples given by the workgroup are: impaired ability to acquire and remember new information; impaired reasoning and handling of complex task and poor judgement; impaired visuospatial abilities (like inability to recognize faces or

common objects); impaired language functions (speaking, reading, writing); changes in personality or behavior, including uncharacteristic mood fluctuations, apathy and socially unaccepted behaviors [17].

Since the assessment of dementia is complex there are pitfalls in neuropsychological testing both medical and non-medical that may influence a personal performance on a cognitive test. Comorbidity may influence the performance, for instance depression, anxiety and renal failure [16]. Level of education is an important variable that may influence performance and therefor scorings needs to be compared to normative scores; derived from testing of healthy individuals generally controlled for age and education [18].

1.2 Cognitive function, nutrition and lifestyle factors

1.2.1 Cognitive function, dementia and vitamin D

MCI is characterized by declining and disturbance of cognition, minimal impairment of complex activities, ability to perform regular daily functions, and absence of dementia [15]. The prevalence of MCI is about four-times greater than dementia. Within a year, the conversion rate from MCI to dementia is about 10-15 % among MCI and the conversion rate increases to 80%–90% after approximately 6 years [5].

Dementia is a result of a complex interplay between environment and genetic factors [19]. The most common form of dementia, Alzheimer's disease (AD) accounts for 60–80% of cases [10], the second most common form is vascular dementia (VaD) [20]. Prevention aiming at modifiable risk factors is crucial given that there are no treatments available to stop or reverse any dementia that is neurodegenerative [21, 22]. Modifiable risk factors include lifestyle and nutrition [23, 24]. There is a growing interest in vitamin D (blood serum 25-hydroxyvitamin D (25OHD)) because of its potential influence on cognitive function among older adults [25]. Older adults are at a higher risk for 25OHD deficiency [26] than younger people and several studies have reported a positive association between 25OHD and cognitive function among older adults [27-29]. Deficient 25OHD concentrations have also been detected among community-dwelling older adults, diagnosed with MCI [30]. A recent longitudinal study with a 12-year follow-up showed a twofold risk for developing all-cause dementia in a nondemented population when participants were either deficient or insufficient in vitamin D [31]. Among participants with a mild stage of dementia, the same study reported an almost triple risk for a progression to a more severe stage of dementia for participants with deficient (<25 nmol/L) vitamin D as compared to those with sufficient vitamin D. In a retrospective

study analysing the impact of vitamin D treatment in the progression of Alzheimer's disease, results showed that the time of progression to a more severe stage, was slower among those who were treated with vitamin D [32]. However, results have been controversial for the association between vitamin D and cognitive function mainly because of the study design, the different cut-off points for vitamin D status classification, and different diagnostic criteria for the cognitive function which make it difficult to compare these studies [33]. Although cross-sectional studies more often yield positive associations [34] as compared to prospective cohort studies, they cannot answer the question of whether vitamin D deficiency leads to cognitive decline, the results from cross-sectional studies need to be supported with prospective studies and randomized clinical trials (RCT) [35].

1.2.2 Vitamin D and the biological basis

Vitamin D is synthesised through the exposure of the skin to solar UV-B radiation [36]. The active form of vitamin D is a hormone that comes in two different forms: One is derived from dietary supplements (vitamin D_2, also known as ergocalciferol). The other is derived from animal food and synthesis from the skin (vitamin D_3, also known as cholecalciferol) [37]. The synthesis of cholecalciferol through sun exposure is the major natural source of vitamin D. Cholecalciferol is converted in the liver to calcifediol (25-hydroxy–cholecalciferol); ergocalciferol is converted to 25-hydroxyergocalciferol. These two vitamin D metabolites (called 25-hydroxyvitamin D or 25OHD) are measured in serum to determine a person's vitamin D status. Calcifediol is further hydroxylated by the kidneys to form calcitriol (also known as 1,25-dihydroxycholecalciferol), the biologically active form of vitamin D [38]. The reference value for low vitamin D levels are generally considerate to be deficient if <30 nmol/L and insufficient when 30-50 nmol/L [39].

To understand why individuals might have insufficient levels of vitamin D, it is important to dive into the literature, especially given the fact that there are several personal and environmental factors that can help or hinder the first step of the synthesis. The first and perhaps the most important factor is age, which can mitigate the body's ability to produce the important active form of vitamin D [40].

Other factors that differently influence serum vitamin D levels include clothing, sunscreen usage, working environment, outdoor physical activity, sun exposure availability, season, latitude (Iceland ~64°), time of day, pollution and ozone, amongst others [33]. Age is a considerably important factor since studies show that vitamin D deficiency is likely to be high among older adults

because the skin, liver and kidneys lose their capacity to make and activate vitamin D with advancing age [37]. Older adults also spend most of their days indoors, and when outside they tend to cover up the skin with protective clothing or apply sunscreen to avoid sunlight exposure [41].

Scientists have discovered that many tissues respond to vitamin D including cells in the immune system, pancreas, skin, and cartilage, reproductive organs as well as brain and nervous system [42]. The vitamin D receptors (VDRs) in the human brain were first reported in a study by Eyles et al. (2005) and confirmed that they were localized in both neuronal and glial cells [43]. Most of the biological actions of vitamin D are exerted through vitamin D receptors acting as a ligand for the active form of vitamin D (1,25 -dihydroxy vitamin D3 receptor) [44].

Studies have shown that vitamin D possesses neurosteroid properties that might be involved in brain health [45], which include regulations of calcium homeostasis, clearance in amyloid beta-peptide (amino acids that are crucially involved in Alzheimer's disease), antioxidant and anti-inflammatory actions. Accumulating evidence from epidemiological and clinical studies has suggested a connection between vitamin D and blood pressure which is one of the modifiable risk factors associated with vascular dementia [46]. In accordance with this knowledge, the interest in the association between vitamin D and cognitive function has been growing over the years.

1.2.3 Cognitive function and physical activity

Epidemiological studies suggest that regular physical activity is closely related to the health of older adults [47-50]. Physical activity is an important determinant of active aging and has a major role in improving the quality of life [51-53]. Physical activity generally decreases with age, and studies have shown that many older adults are insufficiently active when compared to current physical activity recommendations [54-57]. Benefits from physical activity include a reduced risk of cardiovascular disease, hypertension, stroke, type 2 diabetes, obesity, osteoporosis, some forms of cancer as well as improvements in aspects of mental health [58, 59].

Emerging evidence suggests that physical activity can improve cognition in people without dementia, reduce the incidence of dementia [60], and improve health among people with existing dementia [61]. Cross-sectional studies have suggested that individuals who have been diagnosed with MCI have lower rates of physical activity and higher sedentary behaviour [62]. Studies have suggested that physical activity sustains cerebral blood flow by decreasing

blood pressure, lowering lipid levels and by inhibiting platelet aggregation which presumable might be an underlying factor in this association to cognitive function [63]. Clinical trials of both aerobic and resistance training show positive effects on executive function, attention, and processing speed, with inconsistent evidence for memory and other domains [64, 65] among healthy older adults.

Physical activity is one of several modifiable factors that may have relations to the speed and magnitude of cognitive decline experienced with age [66, 67]. In epidemiological studies, health relationships can be considered as a web of causation, and it is critical to consider an array of potential measured and unmeasured risk factors. This was highlighted in a study by Cohen et al. (2017) were physical activity was shown to mediate the effect of body mass index with cognitive function, lower levels of physical activity were shown to contribute to higher body mass index and poorer cognitive function [58].

Cognitive dysfunction among obese older adults has frequently been reported [68]. In the current study, we investigate the association between body weight changes with three specific cognitive function domains (executive functions, memory, and processing speed), which are addressed in paper III.

1.2.4 Cognitive function and body mass index

Aging is associated with decreasing body mass index [69]. The relationship of body mass index and mortality follows a U-shaped curve, with persons at either end of the spectrum, with low or high body mass index, having an increased risk of death [68]. According to a recent meta-analysis study by Lee et al. (2020) individuals who were underweight (<18.5 kg/m^2) or obese (≥30.0 kg/m^2) were at increased vascular dementia risk. The study, pooled data from 19 prospective cohort studies and four clinical trials, distinguishing 5 categories of body mass index. Compared with the reference group (lower-normal weight: 18.5-22.4 kg/m^2), those who were underweight had an approximate 80% greater dementia risk, and for those who were obese, the risk was approximately 50% higher [70]. However, there are still inconclusive results on the associations between obesity and dementia. According to a recent meta-analysis, currently available evidence does not fully support an association between overweight/obesity and incident dementia in old age [71].

There are a few possible explanations for the association of higher body mass index with dementia or other cognitive outcomes. High body fat is associated with increased levels of leptin (a hormone produced by fat cells) which has been suggested to act as a protective factor for cognition in old age [72]. The distribution of body fat could also be a possible explanation for the

inconclusive associations between obesity and dementia among older adults. Previous studies reported the association between visceral adipose tissue, microstructural brain tissue with poorer brain connectivity [73]. On the other hand, a higher amount of subcutaneous fat was negatively associated with the risk of dementia in a cross-sectional study [74]. Visceral fat might be a driving force in these associations between weight gain and dementia. On the other hand, weight gain among older adults can also be associated with a sedentary lifestyle and physical inactivity [58].

Fewer studies are available on changes in body weight and cognitive function among older adults. It has been shown that weight loss is associated with the risk of dementia, although weight loss might rather be a consequence of the preclinical phase of dementia [75] suggesting reverse causation between weight loss and dementia. Therefore, the associations between cognitive function and weight changes are less clear.

The mechanism regarding weight loss and brain activity is not fully understood, but recent studies have suggested that apathy, anxiety, depression and irritability among dementia and MCI cases affect appetite [76]. However, it is also conceivable that weight loss could accelerate brain atrophy before the onset of MCI or dementia. Weight loss can be caused by an inadequate dietary intake, which leads to a deficiency in critical nutrients.

1.2.5 Cognitive function and other modifiable risk factors

Dementia is a result of disease processes which usually develop over several decades and there are multiple causes that both can influence the risk (from mid-life approach or even life-time approach) and the diagnosis of the disease. Diagnosis of dementia should consider that cognitive dysfunction can be due to reversable causes including sleep deprivation, stress, drug and alcohol use, depression, thyroid disease and vitamin deficiency [77].

Evidence for lifestyle at mid-life and subsequent dementia risk has been demonstrated [24]. However, lifestyle in middle age usually reflects lifelong patterns of behavior. Environmental factors from childhood on can have a significant influence on the development of cognitive function, for instance optimal nutrition of the first few weeks of a new-born can improve cognitive and brain development [77].

Potential risk reduction for cognitive decline and dementia has been reported for multiple lifestyle factors. Current smoking has been associated with increased risk of cognitive decline [78, 79]. Small or moderate alcohol consumption has been shown to be associated with higher scores on cognitive

function among older individuals although excessive alcohol consumption has been associated with lower cognitive function and also increased risk of fall [80, 81].

When it comes to modifiable risk factors the most consistent evidence is available for the years of formal education, people with more years of education or greater literacy have lower risk of dementia than those with fewer years [79, 82, 83].

Finally, cognitive training intervention studies have demonstrated improvements in memory among treatment groups when compared to the ones in the control group [84].

1.2.6 Prevention strategies for mild cognitive impairment and dementia

Understanding the true associations between a single lifestyle factor and cognitive function is methodologically challenging. Available data suggests that unhealthy lifestyle behaviors frequently occur in combination, indicating that focusing on one variable is insufficient [85, 86]. More health-conscious individuals often engage in not only one but several healthy behaviors simultaneously, thus investigations focused on finding the multiple dimension of healthy lifestyle that are associated with diseases risk are important. Even though firm conclusions cannot be drawn about the association of any modifiable risk factor to cognitive decline, evidence from observational studies suggest an association with dementia from the risk factors associated with diabetes, mid life hypertension, mid-life obesity, depression, physical inactivity, smoking and low education. According to meta-analyses the most promising strategies for prevention of cognitive decline and dementia, were the elimination of physical inactivity (12.7% of Alzheimer's disease cases prevented), smoking (13.9%, prevented) and low education (19.1% prevented) [87]. The state of the evidence on modifiable risk factors for cognitive decline and dementia risk reduction was further reported in 2015 by the Alzheimer's Associations were existing reviews were evaluated [24]. According to the Associations, strong evidence was available for regular physical activity and management of cardiovascular risk factors (diabetes, obesity, smoking, and hypertension) in reducing the risk of cognitive decline and dementia. They further added lifelong learning and/or cognitive training to their model of reduced risk of cognitive impairment and dementia. Studies indicate that health prevention should start at least from mid-life [10, 67, 88, 89] and observational studies generally have reported findings indicating that "what's good for your heart, is good for your brain" [88, 90]. Mid-life data including physical activity, body mass index, and

hypertension are associated with brain health in later life. There is even reasoning for dementia prevention to be assigned as a life course approach prevention. A life course approach underscores the need to study the long-term, complex interplay among genetic, biological and psychological mechanisms from birth [88]. Low education is one of the strongest risk factors for cognitive decline [9] and since childhood cognitive ability and education correlate highly with cognitive ability in mid-life and old age the life-course perspective is well suited. Further, learning during childhood is critical for the development of cognitive reserve [91] in older age. The term cognitive reserve is purported to act as a moderator between pathology in brain and a clinical objective outcome [92]. Interestingly, in a systematic review higher cognitive reserve was estimated to decrease the risk of developing dementia by 46% [93].

The potential for prevention strategies was highlighted in a study by Norton et al. (2014) where the authors concluded that around a third of all Alzheimer's cases (the most common part of dementia) worldwide might be attributable to potentially modifiable risk factors [9]. The potential for prevention has also been demonstrated in a randomized controlled trial (FINGER) resulting in benefits for cognitive function among older adults with a multidomain approach, more precisely increased performance on executive function, speed of processing and memory function [11].

A critical time frame for health prevention promoting cognitive health can be rationalised through the MCI stage since it is a transitional stage between healthy cognition and dementia, there might be a window of opportunity to intervene [62].

Overall, it is essential to have available accurate local data on the health status of the population at large. In Iceland, there is a need to register the prevalence and incidence of dementia and MCI alongside with some indication on how effective the health care provision is in improving the health status and well-being of people involved. This information should also be available to the general public thus encouraging them to participate individually in maintaining the best possible well-being throughout their lives. With future directions in mind, interdisciplinary collaboration with active policies and health prevention programs will play an important role in promoting the early diagnosis and treatment of dementia and MCI. This could further positively contribute to the physical function and well-being of the older person with dementia and by consequence lead to a positive economic and social impacts.

2 Aims

The main objective of the thesis was to examine the associations between serum vitamin D levels, physical activity, body mass index, and cognitive function among older adults living in Iceland. With a well-characterized AGES-Reykjavik cohort, we were able to examine the impact of lifestyle factors on vitamin D levels in groups with different cognitive status, and further investigate the long-term association of body mass index and cognitive function among older adults.

2.1 Paper I

The aim of study 1 (cross-sectional study) was to examine the cross-sectional associations between serum 25-hydroxy vitamin D and cognitive function. A specific aim was particular considerations for the mediating effects of physical activity.

2.2 Paper II

The aim of study 2 (cross-sectional study) was to examine the associations between lifestyle and 25OHD according to three cognitive status among old adults (i.e. normal cognitive status, mild cognitive impairment, and dementia).

2.3 Paper III

The aim of study 3 (longitudinal study) was to examine the effect of body weight changes on the three different domains of cognitive function among participants with normal cognitive function at baseline. Further, the study examined the longitudinal associations between body weight changes and the development of either MCI or dementia.

3 Materials and methods

3.1 Study population

The AGES-Reykjavik study (AGES I-*baseline study*) in general examined risk factors for diseases and disability in old age that includes environmental factors, genetic susceptibility and their interactions. The AGES–Reykjavik study is a continuation of the Reykjavik Study from the Icelandic Heart Associations (IHA). The Reykjavik Study was initiated in 1967 and included men and women born in 1907–1935 living in the Reykjavik area. During 2002 – 2006, 5764 persons were chosen randomly from the survivors of the Reykjavik Study cohort and re-examined for the AGES–Reykjavik study. Detailed baseline information has been described in the AGES-Reykjavik study paper [94].

Between 2007-2011, all surviving AGES I participants (58%, N = 3316) returned for a 5-year follow-up visit (AGES II-*follow-up study*). In AGES I-AGES II, participants underwent a clinical examination and completed questionnaires and a cognitive test battery. Details on the study design and the baseline AGES-RS assessments have been given elsewhere.

In paper 1**,** 5519 had measurements of serum 25OHD, and 4699 of those had complete data on cognitive function. All subjects with dementia diagnosis (n=180, 3.1%) and APOE genotypes ε2/4 (n=115, 2.0%) were excluded from analysis. Participants with APOE genotypes 2/4 were excluded since the allele ε 2 and ε 4 have opposite effects on the risk for cognitive impairment and dementia [95]. The final sample having complete data, included 4304 participants

In paper 2, of those 5519 having measurements of 25OHD, 5512 had data with complete assessment of cognitive status. Participants were categorized into three groups according to cognitive status. After excluding 350 participants with missing values on covariates, the number in each cognitive status group was 4363 (83.8%) with normal cognitive, status, 492 (9.5%) with mild cognitive impairment and 307 (5.9%) with a dementia diagnosis after data cleansing. Therefore, the final analytical sample included 5162 participants.

In paper 3, from the original sample size of 5764, 3316 participants completed the follow-up measurements. Participants with an MCI (n = 204) or dementia diagnosis (n = 47) at baseline and participants having incomplete data (n=445) were excluded from the present analysis. From the remaining sample, 2620 participants had a complete data set of relevant variables and were thus included into the study.

3.2 Outcome measures

3.2.1 Cognitive function assessment

The neuropsychological assessment in the AGES-Reykjavik study, was done by a team composed of geriatricians and a neuropsychologist, who were responsible for the design of the cognitive function domains, the diagnosis of MCI and diagnosis of dementia. For each and every test on cognitive function there was a strict quality control. After each administration of a test, the trained administrators evaluated the quality of the data, flawed test scores before data analysis were eliminated.

Assessment of cognitive function included multiple tests, both at baseline and follow-up, focusing on three cognitive domains, i.e., memory, processing speed and executive function, similarly to investigators in other population-based studies [96, 97].

For each of the domains, a composite score was constructed based on a theoretical grouping of the tests and by converting raw scores into standardized z scores, and averaging the z scores across the tests in each composite, reflecting the distribution within the study sample as previously described [65].

The memory composite measure included the immediate and delayed-recall portions of a modified version of the California Verbal Learning Test [98]. The processing speed composite measure included the Digit Symbol Substitution Test [99], the Figure Comparison Test [100] and the Stroop Test Part I (reading) and Part II (colour naming) [101]. The executive function composite measure included the Digits Backward Test [99], a shortened version of the CANTAB Spatial Working Memory test [3], and the Stroop Test, Part III (word-colour interference).

A confirmatory factor analysis, the results of which have been previously reported, showed that the fit of the composites scores was good [102]

Results on cognitive function from the AGES studies as assessed in the present study have been published previously by several author groups [102, 103, 104, 105].

The three domains of memory, processing speed and executive function composite measures were either used as continuous variables or categorized into low (lower 50%) and high (higher 50%) making each domain a binary variable.

Neuropsychological tests

All the employed tests have been in use in both research and clinical work for several decades. They are well accepted and have been refined over the years. Some of the tests have been modified for the AGES-studies, in order to be able to limit interview length. The inter-rater reliability for all tests was excellent (Spearman correlation coefficients range 0.96–0.99).

The Digit Symbol Substitution Test (DSST)

It has been reported that the DSST is a valid and sensitive measure of cognitive dysfunction impacted by many domains. Performance on the DSST correlates with real-world functional outcomes (e.g., the ability to accomplish everyday tasks) and recovery from functional disability in a range of psychiatric conditions including schizophrenia and major depressive disorder. Importantly, the DSST has been demonstrated to be sensitive to changes in cognitive functioning and offers promise as a clinical decision-making tool for monitoring treatment effects in disorders affecting cognition [106].

California Verbal Learning test (CVLT)

The CVLT is a measure of episodic verbal learning and memory, and demonstrates sensitivity to a range of clinical conditions.[2] The test does this by attempting to link memory deficits with impaired performance on specific tasks. It has considerable support in the neuropsychological literature due to its construct validity. The test-retest reliability of the CVLT has demonstrated stability over time in healthy adults. The reliability ranges from 0.68-0.94 [107-109].

Digit Forward and Backward

The Digit Span Task measures executive function. It assesses one's ability to hold information in short-term memory and manipulate that information to produce some result.

The Digit Span subscale (Digit Span Forward, Backward, and Sequencing combined composite) internal inconsistency reliability has been reported at .093 [110].

Repetitions of DF were correlated significantly ($p < .01$) with Standard Progressive Matrices performance. Repetitions of DB were correlated significantly with performance on the Peabody Picture Vocabulary Tests-Revised ($p < .02$), although a stronger correlation was obtained between DB and SPM performance ($p < .001$) [111].

Figure comparison

The figure comparison test in AGES-Reykjavik was similar to the known and validated pattern comparison test [100, 112]. The test is sensitive for speed of processing (SP) and is sensitive for susequent deficits in other cognitive domains especially working memory [100, 113] and executive function [113]. SP follows a well-defined trajectory over the lifespan with performance increasing throughout childhood and adolescence, then peaks in early adulthood and declines throughout adulthood and older age [114]. SP is sensitive for dementia, particularly Alzheimer´s disease and vascular dementia.

The participants were required to compare two small figures and indicate whether they where similar or dissimilar indicated with the letters E for alike (eins), and Ó for different (ólíkt) with a maximum time of thirty seconds per sheet. The immediate test-retest correlations have been reported previously and ranges from 0.6-0.73 [100, 112].

Cambridge Neuropsychological Test Automated Battery (CANTAB)

Originally developed at the University of Cambridge, the Cambridge Neuropsychological Test Automated Battery (CANTAB) includes highly sensitive, precise and objective measures of cognitive function, correlated to neural networks. CANTAB tests have demonstrated sensitivity to detecting changes in neuropsychological performance and include tests of working memory, learning and executive function; visual, verbal and episodic memory; attention, information processing and reaction time; social and emotion recognition, decision making and response control [115]. A recently published study verified the construct and concurrent validity of the executive function subtests of the CANTAB [116].

Correlations found between CANTAB and traditional neuropsychological test batteries commonly used support the criterion validity of CANTAB. Targeted cognitive domains previously documented to be assessed by CANTAB in other populations have shown to be targeted also in our Norwegian sample. This strengthens the view that CANTAB is applicable for assessment of Norwegian patients in the same manner as English-speaking patients [117].

Modified Stroop test part I (Word reading); Stroop test part II (colour naming); Stroop test part III (Word colour interference)

The Stroop Test is a measure of selective attention and response inhibition. The general principle underlying the task is that in one condition, the individual

reads aloud a list of colour names in which no name is printed in its matching colour. In another condition, the individual names the colour ink in which the colour names are printed. The measure is based on the observation that time to complete the task increases significantly when the individual is asked to name the colour of the ink rather than read the word.

Word and Color scores were found to have excellent classification accuracy, whereas Color–Word yielded acceptable classification accuracy. Sensitivity and specificity values are presented for a range of cutoff scores, as are positive and negative predictive values for setting-specific base rates of invalid performance. Performances on the Stroop Color and Word Test, particularly the Word and Color trials, can be useful in detecting invalid performance in a mixed patient and forensic sample. Clinical implications are discussed [118].

3.2.2 Mild cognitive impairment

The diagnoses of MCI was done by a panel of specialists. The criterion was having deficits in memory or one other domain of cognitive function or deficits in at least 2 cognitive domains without being severe enough to cross the threshold for dementia and without loss of instrumental activities of daily living. Cognitive performance on a given domain was evaluated with scoring <−1.5 SDs below a cut-point determined from the distribution of scores in a cohort subsample [119].

3.2.3 Dementia

A consensus diagnosis of dementia made by a team composed of a geriatrician, neurologist, neuropsychologist, and a neuroradiologist was made according to international guidelines from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

Assessment of cognitive function was done following a three-step protocol to identify subjects with dementia. First, the Digit Symbol Substitution test and the Mini-Mental State Examination (MMSE) were administered to the total sample. Participants who scored 23 or lower on the Mini-Mental State Examination or had a raw score of 17 or lower on the Digit Symbol Substitution test were administered a second diagnostic cognitive test battery. Participants who scored 8 or more on Trails B which was the ratio of time taken for “Trails B/Trails A” or had lower than the total score of 19 for the four immediate recall trials of the Rey Auditory Verbal Learning went on to a third step. This step included a neurological test and a proxy interview regarding medical history, social, cognitive, and daily functioning changes of the participant.

3.3 Predictors

In papers I-III the following predictors were used to estimate the associations with cognitive function/ dementia: 25 hydroxyvitamin D, lifestyle and body mass index; body weight changes.

3.3.1 25 Hydroxyvitamin D

The accredited laboratory from the Icelandic Heart Association conducted 25OHD measurements using the Liaison chemiluminescence immunoassay (DiaSorin Inc., Stillwater, Minnesota). The inter-assay coefficient of variation was < 6.5 % when calculated data are from measurements using a frozen serum pool as the control sample and < 12.7 % when calculated data is from measurements using Liaison quality controls. Existing serum 25OHD levels were then standardized according to the international Vitamin D Standardization Program (VDSP) as previously described. For the analyses standardized 25OHD were categorized into 3 groups based on Guidelines for Health Professionals from the National Institutes of Health (2014): deficient (≤ 30 nmol/L), insufficient (31-49 nmol/L), normal-high levels (≥ 50 nmol/L).

3.3.2 Lifestyle

The following lifestyle factors were evaluated based on a health history questionnaire; Current smoking (yes vs. no), current alcohol consumption (yes vs. no), cod liver oil (daily vs. not daily), vitamin D supplements (yes vs. no), fatty fish consumption (< 3 vs. ≥ 3 times/week), and medication (≤ 4 vs. ≥ 5). Participants were asked, how many hours per week they participated in moderate/ vigorous intensity physical activity in the past 12 months. Predefined answers were never, rarely, weekly but <1 hour per week, 1–3 hours per week, 4-7 hours per week and more than 7 hours per week. In final analysis physical activity categories were combined into 1. none, 2. ≤ 3 hours/week or 3. > 3 hours/week.

3.3.3 Body mass index and body weight

Weight and height were measured and body mass index (BMI) was calculated as kg/m^2. Participants were categorized as underweight (baseline BMI less than 18.5), normal weight (BMI 18.5–24.9), overweight (BMI 25.0–29.9) and obese (BMI ≥ 30.0). In Paper III, participants were further categorized into weight stable, weight gain and weight loss if they had lost or gained ≥ 3 kg during follow-up as is considered clinically relevant weight changes [120, 121]

3.4 Covariates

Several covariates were included in this thesis including demographic, anthropometric, lifestyle, laboratory and disease-related variables. Education was categorized into four levels (elementary school, high school, undergraduate, more than undergraduate education). Smoking status was evaluated as ever vs. never smoker. Alcohol consumption was evaluated as either currently consuming vs. not consuming. Cod liver oil consumption (never, less than once a week, 1-6 times a week, daily) and multivitamins (yes/no) was assessed via questionnaire. Leisure-time physical activity was assessed by a self-reported questionnaire. Participants were asked, how many hours per week they participated in moderate/ vigorous-intensity physical activity in the past 12 months. Predefined answer categories were never, rarely, weekly but <1 hour per week, 1–3 hours per week, 4-7 hours per week and more than 7 hours per week. In the final analysis, physical activity categories were combined into 1. none, 2. ≤ 3 hours/week or 3. > 3 hours/week).

Participants were instructed in advance to bring all medication they had used during the preceding two weeks before the clinic visit and were categorized into ≤ 4 medications vs. ≥ 5 medications. Diabetes mellitus was defined by a physician's diagnosis of diabetes, the use of diabetes medication and/or fasting blood glucose of >7.0 mmol/L. Hypertension was defined at baseline by a physician's diagnosis of hypertension, use of hypertension medications and/or blood pressure above 140/90 mmHg.

A high level of depressive symptoms was classified as a score of ≥ 6 on the 15-item Geriatric Depression Scale (29). APOE $\varepsilon 4$ alleles were genotyped on a subsample of 2113 people using standard methods (30).

3.5 Statistical analysis

Statistical analyses were carried out using IBM SPSS version 22.0-26.00 (SPSS, Chicago, IL, USA) and a p-value of $p < 0.05$ was considered statistically significant for all analyses.

3.5.1 Paper I

Differences between participants in the 25OHD or physical activity categories were calculated using a chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables (normally distributed).

The associations between 25OHD and each of the three cognitive domains were examined using logistic regression models with a different degree of statistical correction. Therefore, the z-scores of the three domains were

converted into binary variables (low and high) using the 50th percentile as a cut point. Model 1 included 25OHD categories, age, sex and education; model 2 added physical activity; and model 3 additionally included BMI, medication use, diabetes, hypertension, depressive symptoms, alcohol consumption and smoking status as covariates.

3.5.2 Paper II

Baseline Data

Differences in demographic and health characteristics among the three cognitive status groups were calculated using a chi-square test for categorical variables (with Bonferroni correction) and analysis of variance for continuous variables (with LSD post hoc test), crude and age-adjusted. The level of statistical significance was set at $p < 0.05$ ($p < 0.016$ for the Bonferroni and LSD correction).

Linear regression models

Linear regression models (LEM) were used to address the research questions proposed initially applying the continuous outcome of serum 25OHD levels as the dependent variable throughout all calculations.

First, the differences in serum 25OHD levels were calculated among the cognitive status groups (all cognitive status groups in one model) using the normal cognitive status group as a referent group. Second, the associations between lifestyle and serum 25OHD levels were calculated using stratified analysis, therefore calculating the associations separately in each cognitive status group.

The differences in 25OHD levels among cognitive status groups *unstratified analysis*

The differences in serum 25OHD levels among the three cognitive status groups were examined, by calculating the changes in beta between dementia group and the MCI group as compared to the normal cognitive status group. The differences were calculated in 2 linear regression models (general linear model-univariate in SPSS), model 1: crude; model 2: additionally, included age, gender, season, education.

The associations between lifestyle and 25OHD- stratified analyses by cognitive status

The initially proposed research question, regarding associations between lifestyle and vitamin D levels, was examined through linear regression models (general linear model-univariate in SPSS), analyses were done separately for all 3 cognitive status groups (dependent variable: serum 25OHD). Statistical correction for age, gender, season and medication was applied.

3.5.3 Paper III

Demographic, anthropometric, lifestyle- and nutritional data, medication use and APOE ε4 genotype variables were used to describe baseline characteristics of the participants according to body weight change categories. We used a chi-square test for categorical variables and ANOVA for continuous variables to test for statistical differences.

To calculate longitudinal associations between changes in body weight and the three domains of cognitive function, univariate general linear models (GLM) were applied controlling for various confounders. For each outcome variable, the following 3-step model was applied: Model 1 adjusted for age, gender and baseline cognitive function. Model 2 additionally adjusted for 25OHD, baseline BMI and physical activity. Model 3 additionally adjusted for marital status, smoking, education, APOE ε4 and medication use.

To calculate whether changes in body weight predict the onset of MCI or dementia, regression analyses were applied controlling for various confounders as outlined for GLM above.

4 Results

4.1 Vitamin D and the associations with cognitive function (Paper I)

The general population cohort in paper I included a total of 4304 participants after applying exclusion criteria and data cleansing.

Deficient and insufficient levels of 25OHD were detected in 8% and 25% participants, respectively, whereas 67% had normal or high levels of vitamin D. At baseline, the three measured domains of cognitive function all showed a significant difference between 25OHD mean levels (Figure 1), as well as categories, where the lowest cognitive scores were seen in the deficient vitamin D group. Detailed demographic and anthropometric characteristics of subjects for paper I are presented in table 1.

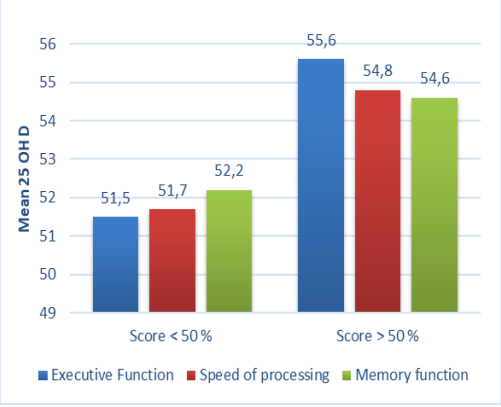


Figure 1 Mean levels of 25OHD according to low vs. high scores on cognitive function domains among cognitively intact men and women

At baseline, all of the disease-related variables (depression, medication use, hypertension and diabetes) were unfavourable related to the deficient group of 25OHD, e.g. individuals who had deficient vitamin D levels also had a higher proporti on of depression, used more medication, a higher proportion of hypertension and diabetes than those who had normal vitamin D levels (> 50 nmol/L). Lifestyle factors were also unfavourable related to the deficient group, e.g. low physical activity and low consumption of cod liver oil/ multivitamin compared to the normal vitamin D group.

Table 1 Demographic and health characteristics according to serum 25-hydroxy vitamin D concentrations among AGES/Reykjavík participants

	Deficient ≤30 nmol/L n = 331 8%	**Insufficient 31-49 nmol/L n = 1058 25%**	**Normal-high ≥50 nmol/L n = 2915 67%**
Female	69%	61%	54%
Age (years)	76.9 ± 6.0	76.5 ± 5.5	76.6 ± 5.6
Elementary education	34%	25%	21%*
BMI (kg/m^2)	27.9 ± 5.4	27.7 ± 5.4	26.7 ± 4.0*
Physical activity			
None	56%	42%	31%*
≤3 h/week	39%	49%	54%*
>3 h/week	5%	9%	15%*
Geriatric depression score (≥6)	8%	7%	5%*
Medication (≥5)	45%	41%	39%*
Hypertension[1] (yes)	84%	84%	79%*
Diabetes[2] (yes)	17%	13%	11%*
Smoking (yes)	16%	12%	7%*
Daily cod liver oil intake (yes)	27%	42%	69%*
Multivitamin use (yes)	14%	22%	36%*
Alcohol consumption (yes)	49%	59%	66%*
Memory function[3]	-0.24 ± 0.9	0.007 ± 0.9	0.05 ± 0.9*
Speed of processing[3]	-0.21 ± 0.9	-0.05 ± 0.9	0.04 ± 0.9*
Executive function[3]	-0.21 ± 0.7	-0.04 ± 0.7	0.04 ± 0.7*

Data are shown as mean ± SD or as %; * Significant differences between the three 25OHD categories according to chi-square test for categorical variables and analysis of variance for continuous variables; 1. Hypertensive: systolic BP > 140 mmHg, diastolic BP > 90 mmHg or medication for hypertension; 2.. Diabetes mellitus was defined by physician's diagnosis of diabetes or use of diabetes medication; 3 Standardized composite score

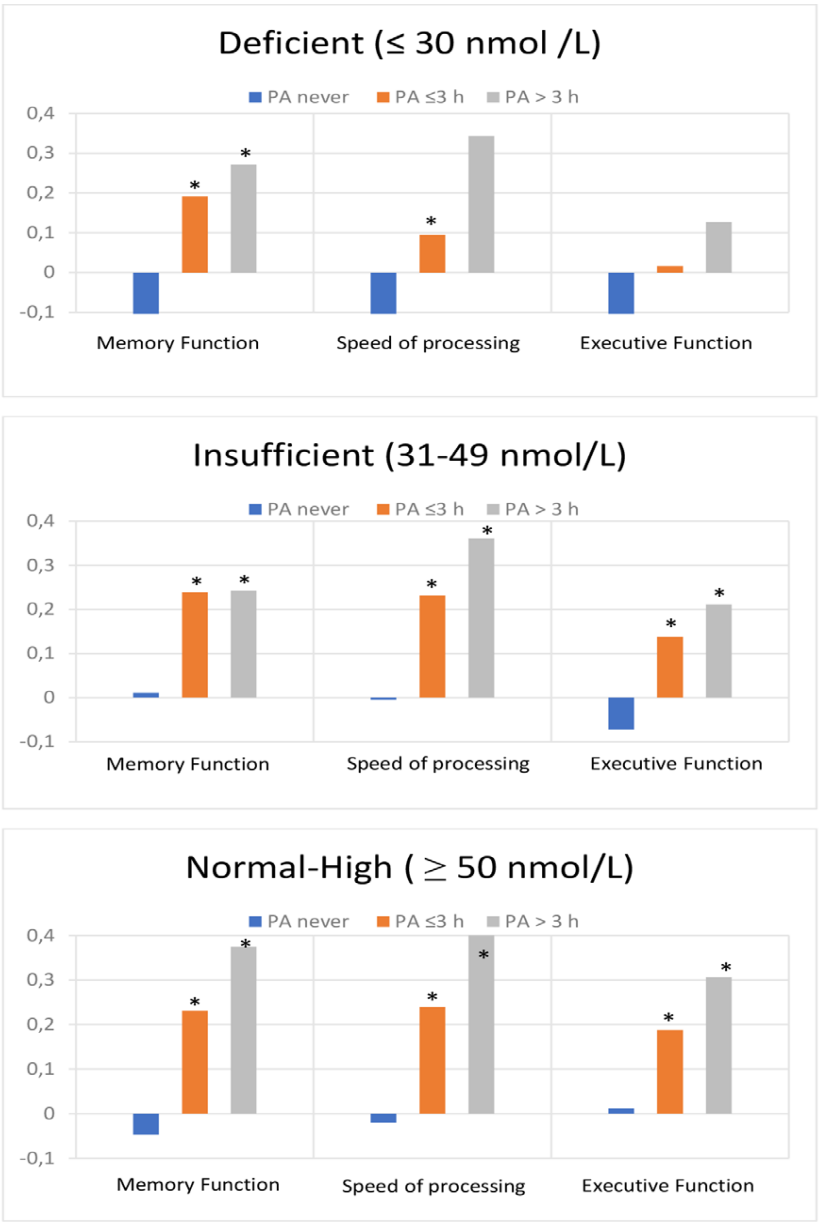


Figure 2 Standardized composite scores of each cognitive domain according to three categories of leisure-time physical activity (PA); categorized as never, ≤ 3 hours/week or > 3 hours/week). The figures are stratified by three categories of serum 25OHD levels. *

Physical activity was a focal point in this study. Sensitivity analyses showed that the associations between scores on the three domains of cognitive function and physical activity, stratified by groups of 25OHD (deficient; insufficient; normal/high) were higher among participants who had higher physical activity if their levels of vitamin D were either insufficient (30-50 nmol/L) or normal. In the deficient vitamin D group, the associations between physical activity and scores on cognitive function were less clear (figure 2).

The final calculations were applied through logistic regression with exponential covariate adjustment. The differences in odds gradually diminished with additional covariate adjustment and thus the greatest differences between the three 25OHD categories were observed in the least corrected model 1. In model 2, an additional adjustment was applied by adding solely physical activity into the calculations leaving the odds ratios for high function marginally changed in the deficient group, e.g., from 0.58 to 0.61 for speed of processing. The changes were similar to the other domains.

In the fully corrected models the odds ratio for the high function of the deficient group remained significantly lower for speed of processing (OR: 0.74, CI: 0.57-0.97) and memory function (OR: 0.61, CI: 0.47-0.80), but not in executive function (OR: 0.76, CI: 0.58-1.00) (Table 2).

Additional calculations were performed to examine whether the associations between vitamin D and cognitive function were linked with mid-life physical activity. Among participants with normal serum 25OHD levels (> 50 nmol/L), high mid-life physical activity was significantly associated with higher cognitive function, in all 3 domains, after adjustment for multiple confounding variables. Results are shown in supplementary table 1.

Table 2 Odds ratio for high cognitive function dependant on 3 levels of 25-hydroxy vitamin D among AGES-Reykjavik participants

	≥50 nmol/L (Normal-high) *n=2915*	31-49 nmol/L (Insufficient) *n=1058*	≤ 30 nmol/L (Deficient) *n=331*
	N=4304		
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Speed of processing			
Model 1	1.00 (ref)	0.86 (0.73-1.00)	0.58 (0.45-0.75)
Model 2	1.00 (ref)	0.88 (0.75-1.03)	0.61 (0.47-0.7
Model 3	1.00 (ref)	0.93 (0.79-1.09)	0.74 (0.57-0.97)
Executive function			
Model 1	1.00 (ref)	0.84 (0.71-0.98)	0.62 (0.48-0.81)
Model 2	1.00 (ref)	0.86 (0.73-1.01)	0.65 (0.49-0.84)
Model 3	1.00 (ref)	0.90 (0.76-1.06)	0.76 (0.58-1.00)
Memory function			
Model 1	1.00 (ref)	1.00 (0.85-1.16)	0.52 (0.41-0.65)
Model 2	1.00 (ref)	1.02 (0.87-1.19)	0.55 (0.43-0.71)
Model 3	1.00 (ref)	1.05 (0.90-1.24)	0.55 (0.43-0.71)

Model I: Adjusted for age, gender, education. Model II: Adjusted for age, gender, education and physical activity. Model III: Adjusted for age, gender, education, physical activity, body mass index, depression symptoms, medication, hypertension, diabetes, current smoking, and alcohol consumption. CI = confidence interval; OR = odds ratio

4.2 Lifestyle and vitamin D in three cognitive status groups (Paper II)

In paper II, the whole study cohort included 5162 participants categorised into three subgroups in the final analyses according to cognitive status, with a total of 307 (dementia), 492 (MCI) and 4363 (normal). As shown in Table 3 most of the baseline characteristics were significantly different between the three groups and in general, the health characteristics of participants with dementia and MCI were worse than among cognitively intact participants. Both, the lowest mean 25OHD and the highest prevalence of vitamin D deficiency were observed among dementia participants, however, the mean 25OHD of this group was still within the normal range. Physical activity levels were low among the dementia group (63% reported no activity), however, the use of supplements was proportionally the highest among the same group (36%).

Table 3 Demographic and health characteristics according to cognitive status among the participants (shown as mean ± SD or as %)

	Dementia (n = 307)	**Mild cognitive impairment (n = 492)**	**Normal cognition (n = 4363)**	**P-value**
Age (years)	81.7±5.5*	80.4±5.7*	75.8±5.2	<0.001
Female	54%*	50%*	59%	<0.001
Education (primary)	34%*	41%*	19%	<0.001
Smoking (yes)	9%	10%	9%	0.51
Alcohol consumption (yes)	48%*	52%*	66%	<0.001
25OHD (nmol/L)	53.8±19.6*	55.8±19.0*	57.6±17.7	<0.001
Deficient (≤ 30 nmol/L)	16%*	12%*	7%	<0.001
Insufficient (30-49 nmol/L)	24%	27%	25%	
Normal (≥50 nmol/L)	60%	61%	68%	
Weekly physical activity				<0.001
No activity	63%*	61%*	44%	
≤ 3 hours	29%	30%	40%	
> 3 hours	8%	9%	16%	
BMI (kg/m^2)	26.3±4.3*	26.8±4.4	27.1±4.4	0.01
Underweight	2%*	2%	1%	
Normal weight	38%	35%	31%	
Overweight	43%	41%	45%	
Obese	17%	22%	23%	
Cod liver oil (yes)	34%*	40%	42%	0.04
Vitamin D suppl. (yes)	36%*	31%	30%	<0.01
Fatty fish (≥3x/week)	11%	12%	14%	0.82
Hypertension[1]	83%	85%*	80%	0.01
Type 2 diabetes[2]	18%*	14%*	11%	<0.001
Medicine count ≥5	58%*	46%*	38%	<0.001
MMSE Score	19.7±5.3*	23.4±3.0*	27.4±2.1	<0.001

*significantly different from the normal cognitive function group
25OHD = 25 hydroxy-vitamin D; BMI = body mass index.
[1] Hypertension, those with systolic BP over 140 mmHg, diastolic BP> 90 mmHg or on hypertensive medication.
[2] Diabetes mellitus was defined by physician's diagnosis of diabetes or use of diabetes medication.

We used linear regression models to detect whether participants with dementia or MCI had significantly lower serum 25OHD levels than cognitively intact participants accounting for several confounders shown in table 4. Subjects with dementia had lower 25OHD levels by around 3.7 nmol/L when compared to subjects with normal cognitive function and neither lifestyle nor medicine use explained this difference.

MCI subjects had lower 25OHD levels in the crude analysis, however, this difference disappeared after statistical correction.

Table 4 Differences in 25OHD levels between the three groups using linear models accounting for potential confounders.

	Model 1				Model 2				Model 3			
	β	95%CI		P-value	β	95%CI		P-value	β	95%CI		P-value
Dementia (n=307)	-3.733	-5.748	-1.717	<0.01	-3.880	-5.968	-1.791	<0.01	-3.450	-5.452	-1.448	0.001
MCI (n=492)	-1.785	-3.429	-0.141	0.033	-1.483	-3.196	0.230	0.090	-0.765	-2.390	0.860	0.356
Normal Cognition* (n=4346)												
Age (years)					0.085	-0.006	0.176	0.067	-0.006	-0.097	0.084	0.891
Male[1]					4.077	3.081	5.074	<0.01	3.254	2.282	4.226	<0.01
Season (summer)[2]					2.824	1.353	4.295	<0.01	4.049	2.503	5.595	<0.01
Education (primary)[3]					-4.284	-6.096	-2.472	<0.01	-0.637	-2.396	1.122	0.478
Physical activity (≥3h/week) [4]									2.597	1.624	3.571	<0.01
BMI (<30 kg/m²)[5]									4.900	3.780	6.021	<0.01
Cod liver oil (daily)[6]									8.827	7.732	9.923	<0.01
Supplements (yes)[7]									5.120	4.116	6.124	<0.01
Fatty fish (≥3x/week)[8]									2.696	1.369	4.023	<0.01
Smoking (no)[9]									4.670	3.093	6.246	<0.01
Alcohol (no)[10]									-2.388	-3.373	-1.402	<0.01
Medicine count (≤ 4)[11]									-0.062	-1.012	0.889	0.899

β = β Coefficient;
*Reference group
Compared to [1] female, [2]winter, [3] university degree, [4] < 3h/week, [5] ≥ 30 kg/m, [6] not daily, [7] no, [8] <3x/week, [9] yes, [10] yes, [11] ≥ 5.
Model 1: crude. Model 2: corrected for age, sex, season and education. Model 3: additionally corrected for lifestyle and medicine use.
as compared to participants with normal cognitive function (n=4363).

Table 5 Associations between lifestyle and 25-hydroxy-vitamin D levels in the three groups of cognitive status.

	Dementia (n=307)			Mild cognitive impairment (n=492)			Normal cognition (n=4363)		
	β	95%CI	p-value	β	95%CI	p-value	β	95%CI	p-value
Physical activity (≥3h/week)	1.77	3.078 6.626	0.47	3.38	-0.45 6.95	0.07	2.82	1.73 3.79	**<0.01**
BMI (< 30 kg/m²)	2.68	-3.134 8.384	0.36	0.99	-2.73 5.32	0.63	5.24	4.43 6.80	**<0.01**
Cod liver oil (daily)	7.12	2.908 12.582	**<0.01**	8.91	5.25 12.79	**<0.01**	9.18	7.99 10.37	**<0.01**
Supplements (yes)	11.52	6.825 15.810	**<0.01**	6.37	3.82 11.01	**<0.01**	4.41	3.32 5.49	**<0.01**
Smoking (no)	0.59	-6.21 7.41	0.86	4.31	-1.17 9.78	0.21	4.79	3.26 6.69	**<0.01**
Alcohol (yes)	2.11	-6.13 2.73	0.32	1.25	-2.07 4.58	0.79	2.67	1.59 3.74	**<0.01**
Fatty fish (≥3x/week)	2.35	3.86 8.56	0.11	4.55	-0.41 9.52	0.07	2.63	1.21 4.10	**<0.01**

Note: β = β Coefficient; BMI = Body mass index.
The statistical model additionally includes age, gender, season and medication.

Throughout the baseline analysis, there were indications in our calculations that those who were either diagnosed with dementia or MCI had also, a somewhat, unfavourable lifestyle. Vitamin D supplementation was though higher among participants who had dementia but at the same time, their serum vitamin D levels were lower in the fully adjusted analysis. Further calculations, following up on the research question, therefore, led to the linear regression analysis, stratified by cognitive status, associating lifestyle with 25OHD levels, the results of these calculations are shown in table 5.

Among subjects with dementia and MCI, cod liver oil intake and vitamin D supplements were the only lifestyle variables significantly associated with 25OHD. Those two variables showed the strongest results in heightening 25OHD levels, ranging from 7.12-9.18 nmol/L (cod liver oil-daily intake) and 4.41-11.52 nmol/L (supplement use).

Among participants with normal cognitive function, all the investigated lifestyle variables were significantly associated with 25OHD.

Among subjects with dementia, there was a similar trend for positive associations between physical activity and 25OHD levels as seen among the normal cognitive function subjects, even though results did not reach statistical significance. The following three variables were associated with the highest 25OHD levels among normal

cognitive function subjects; daily use of cod liver oil, BMI (<30 kg/m^2) and no smoking.

Tables 4 and 5 were further recalculated with random sampling equalizing the 3 cognitive function groups matching the smallest group (n = 307). Random sampling changed the results only marginally and the new calculations were largely in agreement with previous analyses. Results are shown in supplementary table 2.

4.3 Changes in body mass index and the associations with cognitive function, mild cognitive impairment and dementia (Paper III)

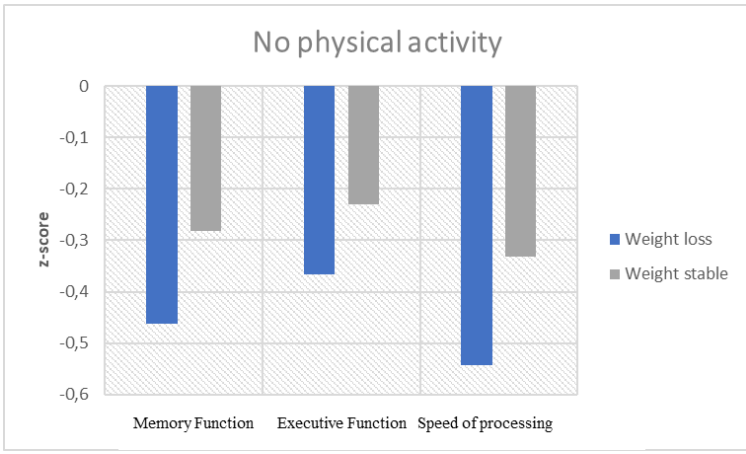
Paper III was a longitudinal study comprising participants from both parts of the AGES-Reykjavik study; AGES-I (2002-2006) and AGES-II (2007-2011). The mean follow-up time was 5.2 years and the cohort comprised 2620 participants who measured up with the inclusion criteria (cognitively intact at baseline). The main aim of this study was to investigate late-life body weight changes and the association with cognitive function/ MCI and dementia. During the follow-up time, 843 participants (32.2%) lost weight (-6.7 ± 3.8 kg), 505 (19.3%) gained weight (5.7 ± 2.9 kg) and 1272 (48.5%) were weight stable (-0.1 ± 1.5 kg). In these categories 83 (9.8%), 33 (6.5%) and 70 (5.5%) participants, respectively, were diagnosed with MCI and 27 (3.2%), 26 (5.1%), 26 (2.0%) participants, respectively, were diagnosed with dementia.

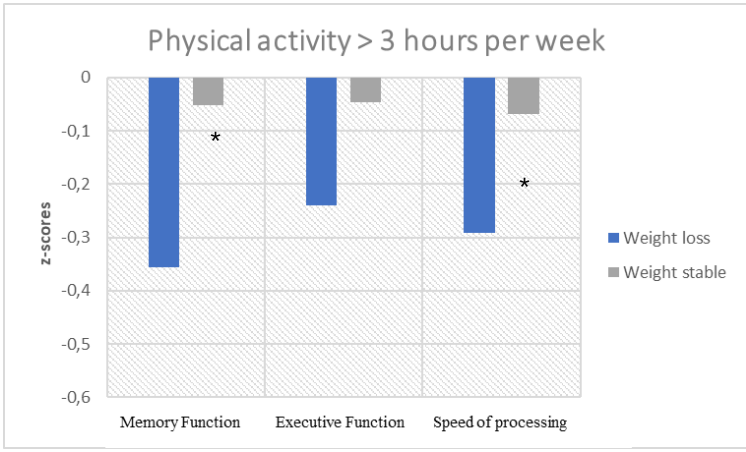
The baseline characteristics of the participants categorized by weight change during follow-up can be seen in Table 6. Most of the baseline characteristics among participants were significantly different between the 3 categories. Participants in the weight loss group had a slightly higher baseline BMI, lower vitamin D levels, fewer were married, and a lower proportion of physical activity. Participants who were in the weight loss category displayed the lowest z-scores in all three cognitive function domains. Even though the weight loss group had lower levels of physical activity, low physical activity did not explain the low cognitive function suggesting that the combination of weight loss and physical activity dose not support higher cognitive function but rather the combination of weight stable and physical activity. Results, for a three-way baseline calculation, (physical activity, cognitive function and body weight changes) are presented in figure 3.

Table 6 Demographic and health characteristics according to weight groups among AGES Reykjavik participants (N = 2620)

	weight loss (n=843)			weight gain (n=505)			no weight change (n=1272)			
	Mean	**±**	**SD**	**mean**	**±**	**SD**	**mean**	**±**	**SD**	**P-value***
Demographic data										
age (years)	75.4	±	4.8	74	±	4.5	74.5	±	4.7	<0.001
male (%)		38			39			44		0.01
female (%)		62			61			56		
education-basic (%)		31.3			23.5			45.2		0.032
married (%)		62.4			61.7			67.6		0.025
Lifestyle data										
physical inactivity (%)		42.0			38.4			35.9		0.008
alcohol-no (%)		32.1			35.5			29.2		0.055
smoke-yes (%)		8.3			10.1			7.8		0.213
Anthropometric data										
BMI (kg/m2)	28.1	±	4.4	27.5	±	4.5	26.6	±	3.9	<0.001
body fat (%)	30	±	7.4	29.6	±	7.7	28.2	±	7.8	<0.001
SBP (mmHg)	143	±	20	140	±	20	141	±	19	0.014
DBP (mmHg)	73.5	±	9.4	75	±	9	74.4	±	10	0.022
Laboratory data										
25OHD (nmol/L)	56.6	±	17	57	±	18.7	59.9	±	16.9	<0.001
Neuropsychological data										
memory (z-score)	0.032	±	0.885	0.173	±	0.890	0.154	±	0.867	0.001
executive (z-score)	0.030	±	0.730	0.060	±	0.762	0.131	±	0.729	0.004
speed (z-score)	0.104	±	0.687	0.061	±	0.705	0.163	±	0.678	0.007
Medication/APOE										
APOE 4 allele carriers (%)		1.9			1.8			1.4		0.589
Medication >5 (number)		35.5			34.4			31.5		0.100

*Chi-square test for categorical variables and ANOVA for continuous variables were to test for statistical differences.





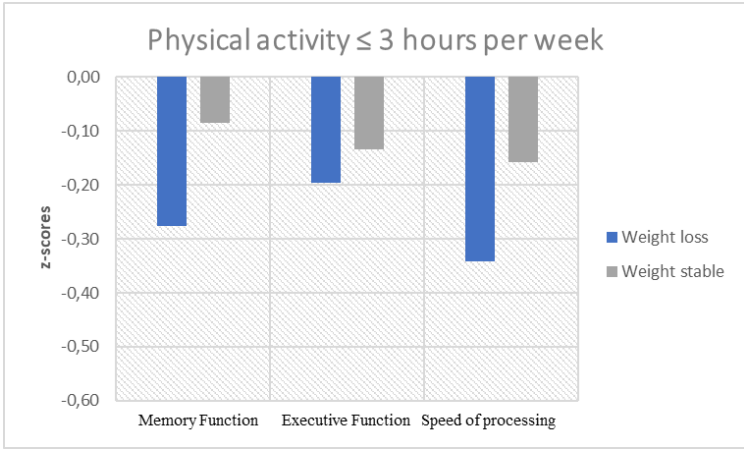


Figure 3 Standardized composite scores (mean) of three cognitive function domains according to weight stable and weigth loss groups. Figures stratified by three categories of physical activity; none, ≤ 3 hours/week or > 3 hours/week. * Significantly different from weight loss according to GLM adjusted for age, gender, education and baseline cognitive function.

To evaluate the longitudinal associations between the weight change categories and scores on the three cognitive function domains, general linear models were applied gradually increasing the statistical correction for confounding factors. Weight loss was associated with a lower memory function and lower speed of processing after follow-up when compared to weight stable. As shown in models 1-3, correction for baseline cognitive function and BMI, demographic factors, lifestyle as well as medication and APOE ε4 variables did only marginally change these results. However, weight loss was not associated with executive function. These results can be seen in tables 7-9.

To predict the longitudinal associations between body weight changes and the development of dementia and MCI, logistic regression was applied showing that weight changes within the cohort were a predictor for the onset of dementia and MCI. Weight loss was associated with a higher MCI risk when compared to weight stable. Further, both weight loss and weight gain were associated with a higher dementia risk when compared to weight stable. Similar to GLM results shown above, correction for baseline BMI, demographic factors and lifestyle as well as medication and APOE ε4 variables did only marginally change these results. The results are shown in tables 10 and 11.

Inclusion of APOE ε4 and 25OHD as covariates did not change the results. Nutritional factors related to vitamin D levels, i.e. cod liver oil consumption and consumption of fatty fish did not have significant associations with any of the cognitive function domains (results not shown in table) and therefore did not alter the associations between body weight changes and cognitive function.

Table 7 Associations between weight change categories and memory function among AGES-Reykjavik

	Model 1				Model 2				Model 3			
Parameter	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
Intercept	2.241	1.805	2.678	<0.001	2.177	1.673	2.682	0	2.15	1.561	2.738	<0.001
weight loss[1]	**-0.097**	**-0.156**	**-0.039**	**0.001**	**-0.097**	**-0.157**	**-0.037**	**0.001**	**-0.098**	**-0.157**	**-0.038**	**0.001**
weight gain[1]	**-0.015**	**-0.085**	**0.054**	**0.666**	**-0.014**	**-0.084**	**0.056**	**0.695**	**-0.009**	**-0.079**	**0.061**	**0.793**
baseline of dependent variable	0.71	0.678	0.741	<0.001	0.71	0.678	0.742	0.000	0.705	0.672	0.737	0.000
male[2]	-0.153	-0.207	-0.098	<0.001	-0.154	-0.209	-0.099	0.000	-0.176	-0.236	-0.117	<0.001
age (years)	-0.032	-0.037	-0.026	<0.001	-0.032	-0.038	-0.026	0.000	-0.032	-0.038	-0.026	<0.001
25OHD (nmol/L)					0.001	-0.001	0.002	0.276	0.001	-0.001	0.002	0.339
BMI (kg/m2)					0.001	-0.006	0.007	0.785	0.001	-0.006	0.007	0.838
physical activity = none[3]					0.024	-0.052	0.100	0.529	0.036	-0.041	0.113	0.357
physical activity = <3 h/week[3]					0.003	-0.069	0.075	0.935	0.003	-0.069	0.076	0.926
married[4]									0.049	-0.067	0.166	0.408
widow[4]									0.038	-0.086	0.162	0.547
divorced[4]									-0.038	-0.192	0.117	0.633
smoking no[5]									0.05	-0.046	0.146	0.308
education basic[6]									-0.082	-0.181	0.018	0.107
education lower[6]									-0.067	-0.15	0.016	0.114
education higher[6]									-0.085	-0.181	0.012	0.086
APOE_4									-0.007	-0.212	0.198	0.947
Medication use < 5[7]									0.027	-0.028	0.083	0.337

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use.[1] compared to weight stable; [2] compared to female; [3] compared to PA>3h/week; [4] compared to single; [5] compared to smoking yes;[6] compared to university;[7] compared to medication use ≥ 5;

Table 8 Associations between weight change categories and speed of processing among AGES-Reykjavík participants (N = 2620)

	Model 1				Model 2				Model 3			
Parameter	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
Intercept	1.396	1.038	1.754	<0.001	1.442	1.028	1.856	<0.001	1.293	0.813	1.773	<0.001
weight loss[1]	**-0.099**	**-0.147**	**-0.052**	**<0.001**	**-0.092**	**-0.140**	**-0.044**	**<0.001**	**-0.092**	**-0.140**	**-0.044**	**<0.001**
weight gain[1]	**-0.038**	**-0.093**	**0.018**	**0.189**	**-0.033**	**-0.089**	**0.023**	**0.245**	**-0.031**	**-0.088**	**0.025**	**0.276**
baseline of dependent variable	0.945	0.913	0.977	<0.001	0.941	0.908	0.973	<0.001	0.932	0.897	0.967	<0.001
male[2]	-0.081	-0.123	-0.038	<0.001	-0.088	-0.131	-0.045	<0.001	-0.101	-0.147	-0.054	<0.001
age (years)	-0.022	-0.026	-0.017	<0.001	-0.022	-0.027	-0.017	<0.001	-0.023	-0.028	-0.018	<0.001
25OHD (nmol/L)					0.001	-0.001	0.002	0.314	0.001	-0.001	0.002	0.412
BMI (kg/m2)					-0.002	-0.008	0.003	0.349	-0.003	-0.009	0.002	0.197
physical activity = none[3]					-0.032	-0.093	0.029	0.304	-0.030	-0.091	0.032	0.342
physical activity = <3 h/week[3]					0.000	-0.058	0.058	0.996	-0.003	-0.062	0.055	0.910
married[4]									0.034	-0.060	0.128	0.473
widow[4]									0.024	-0.075	0.124	0.630
divorced[4]									-0.008	-0.132	0.116	0.900
smoking no[5]									0.069	-0.008	0.147	0.078
education basic[6]									-0.034	-0.116	0.048	0.416
education lower[6]									-0.037	-0.105	0.031	0.286
education higher[6]									-0.023	-0.100	0.055	0.566
APOE_4									0.210	0.045	0.375	0.013
medication use < 5[7]									-0.018	-0.062	0.027	0.438

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use.[1] compared to weight stable; [2] compared to female; [3] compared to PA>3h/week; [4] compared to single; [5] compared to smoking yes;[6] compared to university;[7] compared to medication use ≥ 5;

Table 9 Associations between weight change categories and executive function among AGES-Reykjavík participants (N = 2620)

	Model 1				Model 2				Model 3			
Parameter	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
Intercept	0.832	0.421	1.242	<0.001	0.91	0.432	1.387	<0.001	1.157	0.602	1.712	<0.001
weight loss[1]	**-0.035**	**-0.091**	**0.021**	**0.224**	**-0.027**	**-0.084**	**0.031**	**0.362**	**-0.031**	**-0.088**	**0.026**	**0.285**
weight gain[1]	**-0.051**	**-0.118**	**0.015**	**0.128**	**-0.047**	**-0.113**	**0.02**	**0.168**	**-0.043**	**-0.11**	**0.024**	**0.205**
baseline of dependent variable	0.661	0.627	0.695	<0.001	0.658	0.624	0.692	<0.001	0.633	0.597	0.669	<0.001
male[2]	-0.103	-0.153	-0.053	<0.001	-0.109	-0.160	-0.058	<0.001	-0.138	-0.192	-0.084	<0.001
age (years)	-0.013	-0.019	-0.008	<0.001	-0.013	-0.019	-0.008	<0.001	-0.014	-0.020	-0.008	<0.001
25OHD (nmol/L)					0.000	-0.001	0.002	0.519	0.000	-0.001	0.002	0.705
BMI (kg/m2)					-0.003	-0.010	0.003	0.269	-0.003	-0.009	0.003	0.315
physical activity = none[3]					-0.024	-0.096	0.048	0.514	-0.003	-0.076	0.070	0.932
physical activity = <3 h/week[3]					0.006	-0.063	0.075	0.856	0.004	-0.065	0.073	0.912
married[4]									-0.009	-0.120	0.102	0.872
widow[4]									0.003	-0.114	0.121	0.956
divorced[4]									-0.052	-0.199	0.095	0.490
smoking no[5]									0.082	-0.009	0.174	0.079
education basic[6]									-0.220	-0.315	-0.124	<0.001
education lower[6]									-0.138	-0.218	-0.058	0.001
education higher[6]									-0.095	-0.186	-0.003	0.044
APOE_4									-0.126	-0.322	0.069	0.205
medication use < 5[7]									0.019	-0.034	0.071	0.489

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use.[1] compared to weight stable; [2] compared to female; [3] compared to PA>3h/week; [4] compared to single; [5] compared to smoking yes;[6] compared to university;[7] compared to medication use ≥ 5;

Table 10 Bodyweight change categories and risk of development of MCI among AGES-Reykjavik participants (N = 2620)

	Model 1				Model 2				Model 3			
Parameter	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
weight loss[1]	**1.855**	**1.322**	**2.603**	**<0.001**	**1.768**	**1.253**	**2.495**	**0.001**	**1.850**	**1.303**	**2.627**	**0.001**
weight gain[1]	**1.424**	**0.920**	**2.204**	**0.113**	**1.373**	**0.885**	**2.130**	**0.157**	**1.302**	**0.832**	**2.039**	**0.248**
male[2]	1.562	1.149	2.122	0.004	1.668	1.220	2.279	<0.001	2.292	1.626	3.231	<0.001
age (years)	1.144	1.108	1.181	<0.001	1.143	1.107	1.181	<0.001	1.139	1.099	1.181	<0.001
25OHD (nmol/L)					0.991	0.982	1.000	0.052	0.992	0.983	1.002	0.106
BMI (kg/m2)					1.007	0.969	1.046	0.722	0.999	0.960	1.040	0.977
physical activity = none[3]					1.259	0.782	2.026	0.344	1.073	0.661	1.742	0.775
physical activity = <3 h/week[3]					1.164	0.728	1.863	0.526	1.187	0.736	1.915	0.483
married[4]									1.150	0.529	2.501	0.724
widow[4]									1.352	0.610	2.994	0.458
divorced[4]									1.643	0.630	4.284	0.310
smoking no[5]									0.725	0.414	1.271	0.262
education basic[6]									8.480	3.711	19.378	<0.001
education lower[6]									4.743	2.140	10.511	<0.001
education higher[6]									1.921	0.767	4.815	0.164
APOE_4									0.764	0.225	2.595	0.666
medication use < 5[7]									0.670	0.487	0.921	0.014

*Based on logistic regression; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use.[1] compared to weight stable; [2] compared to female; [3] compared to PA>3h/week; [4] compared to single; [5] compared to smoking yes;[6] compared to university;[7] compared to medication use ≥ 5;

Table 11 Bodyweight change categories and risk of development of dementia among AGES-Reykjavik participants (N = 2620)

	Model 1				Model 2				Model 3			
Parameter	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
weight loss[1]	**1.463**	**0.838**	**2.552**	**0.181**	**1.426**	**0.812**	**2.507**	**0.217**	**1.517**	**0.858**	**2.684**	**0.152**
weight gain[1]	**3.031**	**1.720**	**5.341**	**<0.001**	**2.972**	**1.680**	**5.255**	**<0.001**	**3.071**	**1.724**	**5.469**	**<0.001**
male[2]	1.027	0.642	1.643	0.911	1.084	0.672	1.749	0.740	1.308	0.779	2.197	0.310
age (years)	1.182	1.129	1.239	<0.001	1.178	1.124	1.236	<0.001	1.182	1.123	1.245	<0.001
25OHD (nmol/L)					0.989	0.976	1.003	0.131	0.990	0.976	1.004	0.162
BMI (kg/m2)					0.988	0.934	1.046	0.686	0.984	0.928	1.044	0.588
physical activity = none[3]					1.054	0.539	2.062	0.878	0.948	0.479	1.878	0.878
physical activity = <3 h/week[3]					0.796	0.401	1.580	0.515	0.803	0.402	1.604	0.535
married[4]									0.496	0.227	1.083	0.078
widow[4]									0.364	0.157	0.844	0.018
divorced[4]									0.294	0.075	1.158	0.080
smoking no[5]									0.742	0.319	1.726	0.488
education basic[6]									5.263	1.748	15.849	0.003
education lower[6]									2.422	0.828	7.083	0.106
education higher[6]									2.449	0.779	7.701	0.125
APOE_4									0.589	0.130	2.670	0.492
medication use < 5[7]									0.918	0.564	1.495	0.731

*Based on logistic regression; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use.[1] compared to weight stable; [2] compared to female; [3] compared to PA>3h/week; [4] compared to single; [5] compared to smoking yes;[6] compared to university;[7] compared to medication use ≥ 5;

5 Discussion

The main findings of the project were that community-dwelling older adults who had deficient levels of 25OHD were significantly less likely to have a high cognitive function as compared to participants with normal-high 25OHD levels. Physical activity itself was significantly associated with cognitive function but did not alter the association between 25OHD and cognitive function.

Community-dwelling older adults with dementia in Iceland were at higher risk for vitamin D deficiency when compared to healthy individuals, although the majority still had vitamin D levels within the normal range. Older people with dementia seem to rely more on vitamin D supplements than their healthy counterparts. Physical activity reported among participants with dementia and MCI was low and not associated with 25OHD levels in these groups. Although participants with dementia and MCI had poorer lifestyles than healthy participants, differences in lifestyle did not fully explain the observed lower levels of 25OHD in the dementia group.

Weight loss was associated with lower cognitive function in all three cognitive function domains in a subset of cognitively normal participants. Weight loss increased the risk of developing mild cognitive impairment. Conversely, weight gain was associated with increased dementia risk although this might partly be explained by lack of physical activity. Sensitivity analysis showed that the risk of developing dementia among older adults who gained weight was only significant among those who did not participate in any physical activity.

5.1 Vitamin D associated with cognitive function

Data gathered in the AGES-Reykjavik study gave us a unique opportunity to study the risk of early changes in cognitive function, measured with multiple acknowledged tests according to serum 25OHD levels among community-dwelling older adults in Iceland. In Paper I, our major findings were that participants who had deficient levels (<25 nmol/L) of 25OHD had lower odds for having a high cognitive function. Compared to those who were >50 nmol/L in vitamin D those who were <25 nmol/L had 26% lower odds for having a high speed of processing, 24% lower odds for high executive function and 45% lower odds for having a high memory function. Our results are in agreement

with several earlier studies, although the present literature is complex with regards to the study design and also, the diversity in measuring cognitive function.

Several epidemiological studies have been published on vitamin D and cognitive function which were recently summarized in a systematic review by van der Schaft et al. [123] This review included 25 cross-sectional studies on vitamin D and cognitive function. In agreement with our study, the main result of the review was a statistically significant worse outcome on one or more cognitive function tests or a higher frequency of dementia with lower vitamin D levels or intake in 18 out of 25 (72%) studies. Importantly, van der Schaft et al. (2013) discussed and analysed adjustment for potential confounders to assess the relationship between vitamin D and cognition. Generally, stronger results have been reported between vitamin D and cognitive function in the field of cross-sectional studies [34].

A recent study (2018) [124] concluded that vitamin D status was associated with executive function a decade later. In this study, there seems to be an association between serum 25OHD > 25 nmol/L and improved aspect of executive function 10 years later. Adjustments for confounding were extensive but there was no statistical correction for baseline cognitive function, results may, therefore, be confounded by reverse causality. A study using Mendelian randomisation approach including 17 cohorts, concluded that there was no evidence for serum 25OHD as a causal factor for cognitive performance observed in mid- or later -life [125]. The study showed that there tended to be a u-shaped association in vitamin D and cognitive function and further speculated that this might be the cause of reverse causation or confounding. A recent longitudinal study with an 18 year follow up time resulted in no associations between baseline vitamin D and dementia nor cognitive impairment, these results from a longitudinal study design have also been confirmed in the latest literature on vitamin D and cognitive function [126].

Even though randomized controlled trials (RCT) are considered the preferred type of study for proving drug efficacy, there might be inconsistency within the study design that dilutes the effect of vitamin D supplementation. An example might be that vitamin D, has a competing factor since participants can raise their 25OHD concentrations from sun exposure while participating in the study, therefore minimizing the effects. Another factor that has been reported in prior trials on vitamin D, is that participants are often within normal vitamin D levels at the study baseline. In the VITAL study, which was a large vitamin D supplementation RCT, half of the participants were already consuming 800 IU

of vitamin D supplements per day. Participants need to be either deficient or insufficient to make it possible to detect any effects from the supplementation [127].

According to the present literature, vitamin D trials measuring subsequent cognitive function have generally not been successful. In a systematic review, three vitamin D supplementation, RCTs were included, with a maximum of 6 weeks length, concluding no significant improvements in cognitive functions [34]. In a 24-week intervention study [128], the authors concluded that vitamin D replacement may not improve cognitive function even though low vitamin D levels were successfully raised to adequate levels among older adults. Finally, a long-term supplementation trial (7.8 years) among older women to prevent cognitive decline, MCI and Alzheimer's disease, showed no benefits for cognitive function. Appropriately designed intervention studies are however needed in the nearest future to support or refute the present literature.

5.2 Low prevalence of vitamin D deficiency in Iceland

In general, our participants had mean levels of serum 25OHD comparable to those reported in other northern latitude countries (32-35).

In Iceland, the latitude seems like a highly influential factor in vitamin D concentrations among older adults. One might even assume that differences in UVB exposure, would be mirrored by differences in the prevalence of vitamin D deficiency but this is not always the case.

There is a variation in mean 25OHD levels across Europe and studies have indicated that there is a positive association between 25OHD and latitude i.e. higher levels have been detected in northern countries than in countries in mid-Europe and even in south Europe [41]. Lips (2007) concluded that this was due to more sun-seeking behaviour and white skin in the north, whereas in southern people tended to avoid sun exposure and were more likely to have pigmented skin [129]. The variations in serum 25OHD concentrations between countries in Europe were further confirmed in a study by O'Neill et al. (2016) where Northern European countries had a lower prevalence of vitamin D deficiency than that in the mid-latitude European countries [41]. The winter minimum serum 25OHD concentration was detected in Tromsø, Norway and Reykjavik, Iceland, showing both of these Northern European populations (54.8 and 56.9 nmol/L, respectively), with much higher mean levels than that of the Irish adult population (40.6 nmol/L). This is despite a much-reduced UVB exposure in these countries and winters with further reduced UVB exposure, of seven and eight months for Reykjavik and Tromsø, respectively. The study also showed

that seasonal variation in serum 25OHD concentration of adults in Reykjavik and Tromsø was more blunted than that seen in the Irish adults. An important explanation exists on that matter. In an Icelandic study, mean levels of 25OHD increased exponentially with age. Vitamin D supplementation and cod liver oil consumption increased in accordance with age. When comparing the oldest age group (70-85 years old) to other age groups, a smaller fluctuation in serum 25OHD levels and higher mean levels associated with both season and supplement intake were detected [130]. The higher vitamin D intake in the Northern countries is likely to facilitate serum 25OHD concentrations with less seasonal fluctuations.

5.3 Lifestyle factors in different cognitive status groups and the associations with vitamin D

Since longitudinal studies have suggested that a healthy lifestyle, e.g., high physical activity, appropriate dietary intake and normal BMI, is associated with sufficient vitamin D levels in the general population the main aim of Paper II was to examine the associations between lifestyle and vitamin D concentrations with considerations for cognitive function. At that time, studies examining the associations between lifestyle and vitamin D status in subjects with MCI or dementia, were not available, therefor calculations were stratified by 1) normal cognitive status, 2) MCI, and 3) dementia, adding a novelty to the literature. There was a strong association between intake of vitamin D supplements, cod liver oil consumption and higher concentrations of serum 25OHD in all three groups. The associations with supplements/cod liver oil were somewhat stronger in dementia and MCI than in the normal cognitive status group, possibly reflecting the higher intake among these two groups. All of the other lifestyle variables had significant associations with 25OHD concentration in the normal cognitive status group, cod liver oil had the strongest associations, body mass index <30 kg/m^2 showed higher concentration by 6.8 nmol/L, compared to ≥30 kg/m^2 and physical activity ≥3 h/week showed a higher concentration by 3.8 nmol/L, compared to <3 h/week.

The second aim of Paper II was to assess lifestyle factors among community-dwelling older adults with normal cognitive function, MCI and dementia. Participants with dementia and MCI had a higher prevalence of physical inactivity which was the most discriminative lifestyle factor when compared to participants with normal cognitive function, 63% and 61%, respectively.

As reported previously [131], we found that participants with dementia were less physically active. Studies suggest individuals with dementia benefit from physical activity measured by overall health and well-being [132]. A study by van der Roest et al. revealed that in 40% of people with dementia, the needs for physical activity were not fulfilled [133].

In our study, the majority of participants with dementia and MCI were physically inactive. Since studies have shown that physically inactive individuals with dementia have increased risk of cardiovascular disease, metabolic aberrations and an accelerating progression of dementia, these results need to be taken carefully into consideration. This might also be an important explaining factor for the lack of positive associations between physical activity and 25OHD concentrations as were seen among the normal cognitive status group.

Also, studies indicate that individuals that have mobility restrictions as a result of cognitive disabilities are an important group of people who often get left behind in intervention studies that promote physical activity [134]. Individuals with dementia or cognitive deficits need to be considered in tertiary preventions which focuses on people who are already affected by a disease, improvements in their quality of life and possibly limiting or even delaying further complications of the disease.

As previously observed [135], BMI was negatively associated with 25OHD, but we could observe this only in the normal cognitive status group and no significant relationship between BMI and 25OHD was observed in the dementia or MCI groups.

In our study smoking prevalence was low in all three groups. In the group with a normal cognitive function, it was related to lower 25OHD by around 4 nmol/L which has been seen before [136]. A negative correlation between 25OHD levels and smoking could be explained by the fact that smoking is usually accompanied by a less healthy lifestyle. However, in our analyses, the difference remained although we corrected for various lifestyle factors.

Participants with normal cognitive function reported more frequent alcohol consumption than the other two groups. For this group, we also observed a positive association between alcohol consumption and 25OHD. Similar results were reported previously in a Finnish cross-sectional study [137]. However, a recently published review article on vitamin D and alcohol consumption found mixed results, indicating that the direction of the association between vitamin D and alcohol depends very much on the investigated population, e.g., alcoholic patients vs. moderate drinkers [138]. Newer experimental evidence supports neither a positive nor a negative causal effect of moderated drinking on 25OHD [139].

5.4 Body weight changes associated with early changes in cognitive function, mild cognitive impairment and dementia

In paper II, we examined the lifestyle factors associations with vitamin D concentration. In our baseline calculations, the mean levels of BMI were not significantly different between the groups in the age-adjusted analysis. Although when examining the BMI levels by groups (dementia/MCI/NCS), a somewhat higher proportion of participants with dementia were underweight and normal weight compared to the group with normal cognitive status. The differences in the BMI levels were small, equalling around 3 kg body weight. Although these differences were small we concluded that they might be of importance, since studies have suggested that older adults who are overweight compared to those who are normal weight show better cognitive performance [140] and weight loss may be a preclinical indicator of Alzheimer disease [141].

In Paper III, we found associations between body weight changes and cognitive function in a study exclusively including participants with normal cognitive function at baseline reducing the risk of reverse causation bias. These results are in agreement with several previous studies on this topic [19, 25, 60, 67, 140, 142].

In our study weight loss during the study period was associated with an 85% higher risk of MCI diagnosis. This is in agreement with a large prospective longitudinal cohort study from the USA in which weight loss was associated with a higher risk of incident MCI independent from BMI [143]. Contrary to our expectations, we found that weight gain during follow-up was associated with a greatly increased risk of dementia. In contrast, two cohort studies from the USA reported weight loss to be associated with a higher risk of incident dementia [144, 145] whereas weight gain did not have any significant associations [145]. No information is available in published literature linking weight gain with dementia risk, although there are several studies published having linked obesity to dementia risk [146, 147].

Physical activity has been shown to have positive implications for various health-related outcomes among older adults, [59, 148, 149], including brain health [67, 150]. The proportion of physical inactivity among the weight gain group was high or 38%. Additional calculations stratifying by physical activity levels showed that the weight gain - dementia associations were mainly driven by participants who did not engage in any physical activity. This further confirms the protective effects of physical activity among this group of older adults. However, being physically active (< 3 hours per week) significantly enhanced both speeds of processing and memory function among participants who were stable in weight but not in those who lost weight (figure 3; supplementary table 3-5).

Strength and limitation

AGES-Reykjavik study is a population-based study that has a proportionally large national representation of older individuals. The participation rate is high (75% among men and 68% for women) and item non-response is very low, the vast majority has responded to all questions. It is a strength that our measurement of 25OHD was a standardized measure since studies failing to utilize standardizations have been criticized and can be a major contributor to confusion surrounding vitamin D status.

It is a strength of the study that we used detailed cognitive assessment that allowed us to examine specific cognitive domains concerning 25OHD. Also, several health-related, socioeconomic, and lifestyle variables were available for our sample, so we could adjust for several important confounders in the statistical analysis.

When it comes to assessing the associations between lifestyle factors and serum 25OHD concentrations, the AGES-Reykjavik data comprised a variety of health-related, socioeconomic, and lifestyle variables so we could adjust for several important confounders in the course of the statistical analysis, strengthening the overall analysis. This part of the study examined the associations among participants with dementia who might be particularly vulnerable to recall bias, it would therefore have been interesting to have an objective measurement of physical activity instead of using the self-report questionnaires to get more profound results and to minimize bias. Studies of cross-sectional design are limited in their nature and cannot differentiate the cause and consequence of an observed association.

In relation to the assessment of body weight changes and cognitive function, it is a strength of our longitudinal study that it included a large number of participants who underwent detailed examinations at baseline and follow-up of the study. A considerable number of covariates were used in the analysis to investigate whether physical activity, nutrition, APOE $\varepsilon 4$ or other demographic factors explained the relationship between body weight change and cognitive function. However, a longitudinal study design is also limited in its nature and has a possibility of bias and confounding effects. Also, since dementia is a hyper-term, representing a broad array of brain diseases we could not distinguish between common subgroups like Alzheimer's disease and vascular dementia limiting precise interpretation of weight changes among older adults.

Future intervention studies should address the question of whether keeping body weight stable in association with sufficient vitamin D levels and perhaps an active lifestyle helps to maintain cognitive function and decreases the risk of MCI and dementia among older adults.

6 Conclusions

The main conclusions that can be drawn from the studies presented are the following:

In our sample of community-dwelling old adults, participants with deficient 25OHD were less likely to have high memory function or high speed of processing. Physical activity was associated with high cognitive function; however, it did not explain the associations between 25OHD and cognitive function. Additional analysis revealed that high vitamin D status appeared to enhance the associations between mid-life physical activity and cognitive function. Maintaining normal vitamin D levels and high physical activity throughout the life course might contribute to a better cognitive function among older adults.

Community-dwelling older adults with dementia in Iceland were at higher risk for vitamin D deficiency when compared to healthy individuals, although the majority still had vitamin D levels within the normal range. Older adults with dementia seemed to rely more on vitamin D supplements than their healthy counterparts. Physical activity reported among participants with dementia and MCI was low and was not associated with 25OHD levels in these groups. Although participants with dementia and MCI had poorer lifestyles than healthy participants, differences in lifestyle did not fully explain the observed lower levels of 25OHD in the dementia group. Our study showed that participants, who lost body weight during the follow-up period, had lower cognitive function after follow-up compared to weight stable or weight gaining participants and consequently these participants had a higher risk of developing MCI. In contrast to our expectations, we found that participants who gained weight during follow-up were at an increased risk for dementia compared to weight stable participants. Participants' categorical BMI levels themselves, were neither related to cognitive function nor the risk of MCI or dementia. We conclude that keeping body weight stable during old adulthood is the best option to maintain cognitive function in old age.

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SERUM 25-HYDROXY VITAMIN D, PHYSICAL ACTIVITY AND COGNITIVE FUNCTION AMONG OLDER ADULTS

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Abstract: *Objective:* To investigate the association between 25-hydroxy vitamin D (25OHD) and cognitive function with particular consideration of physical activity (PA) in Icelandic older adults. *Design:* Cross-sectional study. *Setting:* Iceland. Participants: Old adults aged 65-96. The final analytical sample included 4304 non-demented participants. *Measurements:* Serum 25OHD was categorized into deficient (≤ 30 nmol/L, 8%), insufficient (31-49 nmol/L, 25%) and normal-high levels (>50 nmol/L, 67%). Cognitive function assessments included measurements of memory function (MF), speed of processing (SP) and executive function (EF) all categorized as low and high (divided by 50th percentile). Multivariate logistic regression analysis was used to calculate the odds ratio (OR) for having high cognitive function. *Results:* Serum 25OHD was positively associated with cognitive function. Adjustment for PA and other potential confounders diminished this association only partially. Compared to participants with normal-high levels of 25OHD, those with deficient levels had decreased odds for high SP (OR: 0.74, CI: 0.57-0.97), high MF (OR: 0.61; CI: 0.47- 0.79) and high EF (OR: 0.76, CI: 0.57-1.0). *Conclusion:* Serum 25OHD below ≤30 nmol/L was associated with decreased odds for high cognitive function among community dwelling old adults as compared to those with 25OHD above > 50 nmol/L. Neither PA nor other potential confounders explained the associations between 25OHD and cognitive function. Future studies should explore mechanisms and the potential clinical relevance of this relationship.

Key words: Vitamin D, memory function, speed of processing, executive function.

Introduction

Serum 25-hydroxy vitamin D (25OHD) has been recognized as crucial for maintaining calcium and phosphate homeostasis which is important for bone health (1). Further, epidemiological studies have indicated associations between low 25OHD levels and a variety of chronic illnesses including type 1 and type 2 diabetes (2), autoimmune diseases and liver disease (3, 4) In recent years there has been a growing interest in the potential role of vitamin D in cognitive function (5-9). Older adults are at a higher risk for 25OHD deficiency than younger people (10) and several studies have reported a positive correlation between 25OHD and cognitive function in this vulnerable group (6, 8, 9).

In general, aging is associated with reduction in brain tissue volume with concomitant declines in cognitive function (11). Impairment in cognitive function has also been linked to lifestyle factors, such as physical inactivity in older adults (12, 13). Evidence from physical activity (PA) intervention studies also indicates that PA might have a role in preserving cognitive function among older individuals (14).

Interestingly, low PA has also been associated with low levels of serum 25OHD (15), possibly related to the lack of sunlight exposure. Since several previous studies, that have investigated serum 25OHD and cognitive function, have not used statistical correction for PA (16, 17), it remains unclear whether the association between 25OHD and cognitive function among older adults is direct or whether it is mediated by PA.

The aim of the current study was thus to investigate the cross-sectional associations between 25OHD and cognitive function with particular consideration of the potentially mediating effects of PA using data from the Age Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik), a large population based cohort of older adults living at a northern latitude.

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Subjects and methods

Study population

The AGES-Reykjavik Study (AGES-RS) examined risk factors for diseases in old age, including environmental factors and genetic susceptibility, and their interactions. Briefly, the AGES–RS is a continuation of the Reykjavik Study in Iceland. The Reykjavik Study was initiated in 1967 by the Icelandic Heart Associations and included men and women born in 1907–1935 living in the Reykjavik area [18]. During 2002 – 2006, 5764 persons randomly chosen from survivors of the Reykjavik Study cohort were re-examined for the AGES–RS. Participants completed a questionnaire, underwent a clinical examination, and completed a cognitive test battery. Details on the study design and the baseline AGES-RS assessments have been given elsewhere (19). The study was approved by the National Bioethics Committee in Iceland (approval VSN-00-063), The Data Protection Authority, and by the National Institute on Aging Intramural Institutional Review Board. Written informed consent was obtained from all participants.

Serum 25-hydroxy vitamin D measurement

The accredited IHA laboratory performed 25OHD measurements in batch using unfrozen serum samples and the Liaison chemiluminescence immunoassay (DiaSorin Inc, Stillwater, Minnesota). The inter-assay coefficient of variation was < 6.5 % when calculated data are from measurements using a frozen serum pool as the control sample and < 12.7 % when calculated data is from measurements using Liaison quality controls. Existing serum 25OHD levels were then standardized according to the international Vitamin D Standardization Program (VDSP) as previously described (20). Standardized serum 25OHD was used as categorized variable in statistical analyses based on Guidelines for Health Professionals from the National Institutes of Health (2014) (21). The cut points of serum 25OHD used in this study were as follows: deficient (≤ 30 nmol/L), insufficient (31-49 nmol/L), normal-high levels (≥ 50 nmol/L).

Cognitive function assessment and dementia

Assessment of cognitive function included nine tests, or test components, focusing on three cognitive domains, i.e., memory, processing speed and executive function. For each of the domains, a composite score was constructed based on a theoretical grouping of the tests and by converting raw scores into standardized z scores reflecting the distribution within the study sample. The inter-rater reliability for all tests was excellent (Spearman correlation coefficients range 0.96–0.99) (22).

The memory composite measure included the immediate and delayed-recall portions of a modified version of the California Verbal Learning Test (23) . The processing speed composite measure included the Digit Symbol Substitution Test (24), the Figure Comparison Test (25) and the Stroop Test (26) Part I (reading) and Part II (color naming). The executive function composite measure included the Digits Backward Test (24), a shortened version of the CANTAB Spatial Working Memory test (27)and the Stroop Test, Part III (word-color interference). The three domains of memory, processing speed and executive function composite measures were each categorized into low (lower 50%) and high (higher 50%) making each domain a binary variable.

A consensus diagnosis of dementia made by a team composed of a geriatrician, neurologist, neuropsychologist, and a neuroradiologist was made according to international guidelines from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (28).

Covariates

A number of covariates were included in the present study including demographic, anthropometric, lifestyle, laboratory and disease-related variables:

Education was categorized into four levels (elementary school, high school, undergraduate, more than undergraduate education).

Body mass index (BMI) was calculated as kg/m2. Smoking status was evaluated as ever vs. never smoker. Alcohol consumption was evaluated as either currently consuming vs. not consuming. Cod liver oil consumption (never, less than once a week, 1-6 times a week, daily) and multivitamins (yes/no) were assessed via questionnaire.

Leisure time physical activity was assessed by a self-reported questionnaire. Participants were asked, how many hours per week they participated in moderate/vigorous intensity physical activity in the past 12 months. Predefined answer categories were never, rarely, weekly but <1 hour per week, 1–3 hours per week, 4-7 hours per week and more than 7 hours per week. In final analysis physical activity categories were combined into 1. none, 2. ≤ 3 hours/week or 3. > 3 hours/week).

Participants were instructed in advance to bring all medication they had used during the preceding two weeks before the clinic visit and were categorized into ≤ 4 medication vs. ≥ 5 medication. Diabetes mellitus was defined by a physician's diagnosis of diabetes, use of diabetes medication and/or fasting blood glucose of >7.0 mmol/L. Hypertension was defined at baseline by a physician's diagnosis of hypertension, use of hypertension medications and/or blood pressure above 140/90 mm Hg.

A high level of depressive symptoms was classified as a score of ≥ 6 on the 15-item Geriatric Depression Scale (29). APOE alleles were genotyped on a subsample of 2113 people using standard methods (30). APOE

genotypes were grouped as APOE ε4 carrier (ε3/4, and ε4/4 genotype) and APOE ε4 non-carrier (ε2/2, ε2/3and ε3/3).

Analytical Sample

Of the total cohort (N=5764), 5519 had measurement of serum 25OHD, and 4699 of those had complete data on cognitive function. All subjects with dementia diagnosis (n=180, 3.1%) and APOE genotypes ε2/4 (n=115, 2.0%) were excluded from analysis. Participants with APOE genotypes 2/4 were excluded since the allele ε 2 and ε 4 have opposite effects on the risk for cognitive impairment and dementia[30]. The final sample having complete data, included 4304 participants (Figure 1). Compared with those who were not included in the analytical sample, those included were significantly younger (76.31±5.4 vs. 80.42±6.7 years, $p < 0.001$) and less likely to have diabetes (11.9% vs. 18.4 %, $p < 0.001$), but the two groups did not differ significantly by gender, blood pressure or medication use.

Statistical analysis

Statistical analyses were carried out using IBM SPSS version 22.0 (SPSS, Chicago, IL, USA). Differences between participants in the 25OHD- or PA-categories were calculated using chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables (normally distributed).

The associations between 25OHD and each of the three cognitive domains were examined using logistic regression models with a different degree of statistical correction. Therefore, the z scores of the three domains were converted into binary variables (low and high) using the 50th percentile as a cut point. Model 1 included 25OHD categories, age, sex and education; model 2 added PA; and model 3 additionally included BMI, medication use, diabetes, hypertension, depressive symptoms, alcohol consumption and smoking status as covariates. The level of statistical significance was set at $p < 0.05$.

Results

Deficient and insufficient levels of 25OHD were observed in 8% and 25% of the participants, respectively, whereas 67% had normal-high levels (Table 1). All of the lifestyle-, and disease-related variables were different between the three 25OHD groups. Cod liver oil and use of multivitamin supplements were associated with higher 25OHD.

Figure 2 shows the associations between cognitive domain scores and PA stratified by 25OHD. PA was associated with higher cognitive function in the normal-high and in the insufficient 25OHD category, however, these associations were less clear in the deficient category.

Figure 1
Flow chart

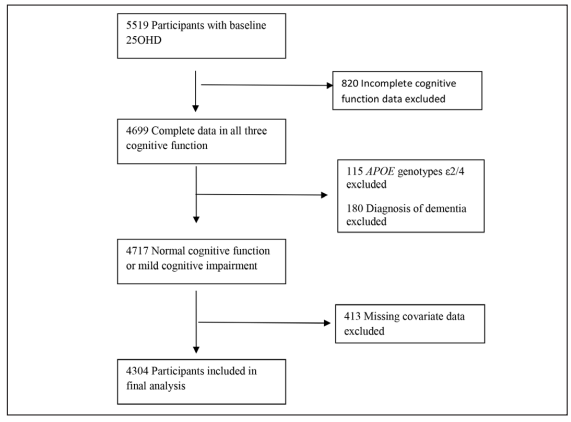
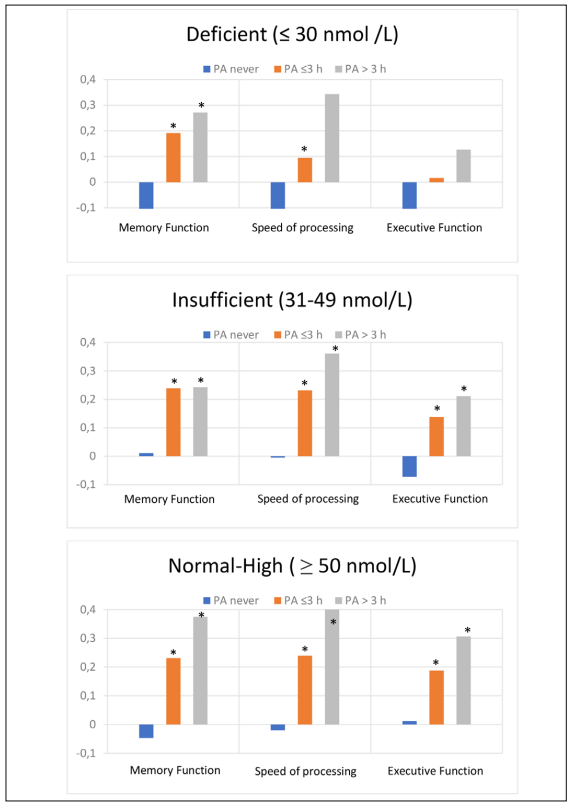


Figure 2
Standardized composite scores of each cognitive domain according to three categories of leisure time physical activity(PA); categorized as none, ≤ 3 hours/week or > 3 hours/week). The figures are stratified by three categories of serum 25OHD levels



*Significantly different from the PA never group.

Table 1
Demographic and health characteristics according to serum 25-hydroxy vitamin D concentrations among AGES/Reykjavík participants

	Deficient ≤30 nmol/L n = 331 8%	**Insufficient 31-49 nmol/L n = 1058 25%**	**Normal-high ≥50 nmol/L n = 2915 67%**
Female	69%	61%	54%
Age (years)	76.9 ± 6.0	76.5 ± 5.5	76.6 ± 5.6
Elementary education	34%	25%	21%*
BMI (kg/m^2)	27.9 ± 5.4	27.7 ± 5.4	26.7 ± 4.0*
Physical activity			
None	56%	42%	31%*
≤3 h/week	39%	49%	54%*
>3 h/week	5%	9%	15%*
Geriatric depression score (≥6)	8%	7%	5%*
Medication (≥5)	45%	41%	39%*
Hypertension[1] (yes)	84%	84%	79%*
Diabetes[2] (yes)	17%	13%	11%*
Smoking (yes)	16%	12%	7%*
Daily cod liver oil intake (yes)	27%	42%	69%*
Multivitamin use (yes)	14%	22%	36%*
Alcohol consumption (yes)	49%	59%	66%*
Memory function[3]	-0.24 ± 0.9	0.007 ± 0.9	0.05 ± 0.9*
Speed of processing[3]	-0.21 ± 0.9	-0.05 ± 0.9	0.04 ± 0.9*
Executive function[3]	-0.21 ± 0.7	-0.04 ± 0.7	0.04 ± 0.7*

Data are shown as mean ± SD or as %; * Significant differences between the three 25OHD categories according to chi-square test for categorical variables and analysis of variance for continuous variables; 1. Hypertensive: systolic BP > 140 mmHg, diastolic BP > 90 mmHg or medication for hypertension; 2.. Diabetes mellitus was defined by physician's diagnosis of diabetes or use of diabetes medication; 3 Standardized composite score

Results from the logistic regression analyses are shown in Table 2. We found that participants with deficient 25OHD levels were less likely (odds ratios between 0.52 - 0.76 depending on the statistical model) to have high cognitive function as compared to participants with normal-high 25OHD levels. Differences between participants with insufficient 25OHD and participants with normal-high 25OHD were not significant in the fully corrected models.

The differences in odds gradually diminished with additional covariate adjustment and thus the greatest differences between the three 25OHD categories were observed in the least corrected model 1. After additional adjustment for PA in model 2, the odds ratios for high function changed only marginally in the deficient group, e.g., from 0.58 to 0.61 for speed of processing. The changes were similar for the other domains.

In the fully corrected models the odds ratio for high function of the deficient group remained significantly lower for speed of processing (OR: 0.74, CI: 0.57-0.97) and memory function (OR: 0.61, CI: 0.47-0.80), but not in executive function (OR: 0.76, CI: 0.58-1.00).

Table 2
Odds Ratio* for high cognitive function dependent on three categories of 25-hydroxy vitamin D among AGES-Reykjavik participants

	≥50 nmol/L (normal-high) n=2915	**31-49 nmol/L (insufficient) n=1058**	**≤ 30 nmol/L (deficient) n=331**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
Speed of processing			
Model 1	1.00 (ref)	0.86 (0.73-1.00)	0.58 (0.45-0.75)
Model 2	1.00 (ref)	0.88 (0.75-1.03)	0.61 (0.47-0.77)
Model 3	1.00 (ref)	0.93 (0.79-1.09)	0.74 (0.57-0.97)
Executive function			
Model 1	1.00 (ref)	0.84 (0.71-0.98)	0.62 (0.48-0.81)
Model 2	1.00 (ref)	0.86 (0.73-1.01)	0.65 (0.49-0.84)
Model 3	1.00 (ref)	0.90 (0.76-1.06)	0.76 (0.58-1.00)
Memory function			
Model 1	1.00 (ref)	1.00 (0.85-1.16)	0.52 (0.41-0.65)
Model 2	1.00 (ref)	1.02 (0.87-1.19)	0.55 (0.43-0.71)
Model 3	1.00 (ref)	1.02 (0.87-1.19)	0.55 (0.43-0.71)

* Odds ratio based on multivariate logistic regression analysis; CI = confidence interval; OR = odds ratio; Model 1: Adjusted for age, gender, education; Model 2: Adjusted for age, gender, education and physical activity; Model 3: Adjusted for age, gender, education, physical activity, body mass index, depression symptoms, medication, hypertension, diabetes, current smoking, and alcohol co

Discussion

In this large cross-sectional study we investigated the associations between 25OHD and cognitive function among older individuals living in Iceland with attention to the potentially mediating effect of PA. We found that participants with deficient levels of 25OHD were significantly less likely to have high cognitive functioning as compared to participants with normal-high levels. PA itself was significantly associated with cognitive function, which has been reported previously. It has been suggested that PA sustains cerebral blood flow by decreasing blood pressure, lowering lipid levels and inhibiting platelet aggregation (31). However, in our study PA did not seem to mediate the associations between 25OHD and cognitive function, as inclusion of PA as covariate in statistical models barely changed the outcomes.

In general, our participants had mean levels of serum 25OHD comparable to those reported in other northern latitude countries (32-35). When looking at the characteristics of our study sample, we found that

participants with deficient 25OHD levels had unhealthier lifestyles and poorer health compared to participants with normal-high 25OHD. They smoked more frequently, had lower engagement in PA and a higher BMI. They also had a higher incidence of depressive symptoms, diabetes, but lower educational levels (Table 1).

For the interpretation of the results it is important to consider that the above mentioned lifestyle and health-related variables can act as confounders. As seen in our calculations, statistical correction for these variables attenuated the relation between 25OHD and cognitive function to a certain degree. However, the association remained significant for memory function and speed of processing and borderline significant for executive function.

There is a biological basis for the role of vitamin D in cognitive function (5). Vitamin D receptors are found in the brain area most vulnerable to aging. They are localized in both neurons and glial cells of the brain (36). In animals, vitamin D deficiency can affect concentrations of neurotransmitters necessary for the normal function of the brain (37) . Further, animal studies have reported that vitamin D supplementation improves learning and memory impairment related to disease (38) or inflammation (39), which are important factors in the ageing process. Finally, vitamin D supplementation prevented cognitive decline in aging rats (37) in an animal model that tried to mimic the range of human 25OHD, i.e., from deficient to normal.

In the past few years several epidemiological studies have been published on vitamin D and cognitive function which were recently summarized in an extensive systematic review by van der Schaft et al. (40). This review included 25 cross-sectional studies on vitamin D and cognitive function. In agreement with our study, the main result of the review was a statistically significant worse outcome on one or more cognitive function tests or a higher frequency of dementia with lower vitamin D levels or -intake in 18 out of 25 (72%) studies. Importantly, van der Schaft et al. (40)discussed and analyzed adjustment for potential confounders to assess the relationship between vitamin D and cognition. There are many potential confounders, including general health, exercise and socio-economic class, of which age, level of education, BMI and gender are the most important ones (40). They found that e.g., around half of the included studies did not adjust for education or BMI. Here is where the present study adds to prior knowledge, because we could show that associations between cognitive function and 25OHD remained significant despite extensive adjustment for potential confounders. However, considering the differences in the majority of health and lifestyle variables between participants in the three 25OHD categories, we cannot exclude the possibility of residual confounding, although the inclusion of a wide range of covariates covering anthropometric, social, psychological, medical and lifestyle variables reduces such a risk.

Being cross-sectional, our study cannot determine whether low 25OHD is a cause or a consequence of low cognitive function. Longitudinal study designs can address this question. The above mentioned review (39) included five prospective cohort studies showing that participants with low 25OHD had faster cognitive decline than those with high 25OHD. This was also shown in a more recently published prospective cohort study by Karakis et al. (41). However, as prospective cohort studies are still sensitive to the effects of confounding, and thus placebo-controlled randomized clinical trials are needed to confirm results obtained from both cross-sectional and prospective cohort studies

Strengths and limitations

It is a strength of the current study that we used detailed cognitive assessment that allowed us to examine specific cognitive domains in relation to 25OHD. Also, several health-related, socioeconomic, and lifestyle variables were available for our sample, so we could adjust for number of important confounders in the statistical analysis. Finally, the final sample size was large, comprising 4304 participants. However, it is an inherent limitation of this study like all other cross-sectional studies that one cannot disentangle cause and effect.

Conclusion

In our sample of community dwelling old adults, participants with deficient 25OHD were less likely to have high memory function or high speed of processing. PA was associated with high cognitive function, however it did not explain the associations between 25OHD and cognitive function.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: AGES-Reykjavik was approved by the National Bioethics Committee in Iceland that acts as the institutional review board for the IHA (approval number VSN-00-063) and the National Institute on Aging Intramural Institutional Review Board. A multistage consent is obtained for AGES–Reykjavik to cover participation and access to administrative records. Release of data for analysis is governed by rules created by these bodies to protect the privacy of Icelandic participants.

Informed consent: Informed consent was obtained from all individual participants included in study.

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Paper II

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ORIGINAL ARTICLE

Lifestyle and 25-hydroxy-vitamin D among community-dwelling old adults with dementia, mild cognitive impairment, or normal cognitive function

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Abstract

Background Several studies have indicated that older adults with cognitive impairment have a poorer lifestyle than their healthy peers including lower 25-hydroxy-vitamin D levels (25OHD).

Aim To investigate the associations between lifestyle and 25OHD depending on cognitive status among old adults.

Methods Community-dwelling old adults (65–96 years) participated in this cross-sectional study based on the Age-Gene/Environment-Susceptibility-Reykjavik-Study. The analytical sample included 5162 subjects who were stratified by cognitive status, i.e., dementia ($n=307$), mild cognitive impairment (MCI, $n=492$), and normal cognitive status (NCS, $n=4363$). Lifestyle variables were assessed and 25OHD was measured. The associations between lifestyle and 25OHD were calculated using linear models correcting for potential confounders.

Results According to linear regression models, 25OHD was significantly lower in older people with dementia (53.8 ± 19.6 nmol/L) than in NCS participants (57.6 ± 17.7 nmol/L). Cod liver oil (7.1–9.2 nmol/L, $P<0.001$) and dietary supplements (4.4–11.5 nmol/L, $P<0.001$) were associated with higher 25OHD in all three groups. However, physical activity ≥ 3 h/week (2.82 nmol/L, $P<0.001$), BMI <30 kg/m^2 (5.2 nmol/L, $P<0.001$), non-smoking (4.8 nmol/L, $P<0.001$), alcohol consumption (2.7 nmol/L, $P<0.001$), and fatty fish consumption $\geq 3x$/week (2.6 nmol/L, $P<0.001$) were related to higher 25OHD in NCS only, but not in participants with dementia or MCI.

Discussion Older people living in Iceland with dementia are at higher risk for 25OHD deficiency when compared to healthy individuals. Physical activity reported among participants with dementia, and MCI is low and is not significantly associated with 25OHD.

Conclusions Lifestyle factors among NCS participants are associated with 25OHD levels. Importantly, healthy lifestyle should be promoted among individuals with MCI and dementia.

Keywords Cognitive impairment · Healthy aging · Vitamin D · Physical activity · Dementia

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Introduction

Vitamin D affects bone health and low levels of 25-hydroxy-vitamin D (25OHD) have been associated with disorders such as osteoporosis and osteomalacia in old adults [1]. In recent years, studies have suggested associations between 25OHD and several health-related outcomes beyond bone health [2]. The presence of vitamin D receptors [3] in many body tissues supports the evidence linking vitamin D deficiency to increased disease risk, e.g., cardiovascular disease, type 2 diabetes, and more recently, to neurodegenerative diseases [4].

Longitudinal studies have suggested that a healthy lifestyle, e.g., high physical activity, appropriate dietary intake, and normal body mass index (BMI), is associated with sufficient vitamin D levels in the general population [5, 6]. Further, several studies have indicated that older adults with mild cognitive impairment or dementia have a poorer lifestyle than their healthy peers, i.e., they are less physically active [7–9] and have lower vitamin D intake [10–12]. Additionally, they do not always get the appropriate health service with respect to dementia [13] and consequently, there is a reason to believe that these individuals are at an increased risk for vitamin D deficiency.

A recent longitudinal study with a 12-year follow-up time, showed a twofold risk for developing all cause dementia in a nondemented population, when participants were either deficient or insufficient in vitamin D. The same study reported, among participants with mild stage of dementia, almost a triple risk for a progression to a more severe stage of dementia if participants were deficient (< 25 nmol/L) in vitamin D as compared to sufficient levels [14]. Also, in a retrospective study, analyzing the impact of vitamin D treatment in the progression of Alzheimer's disease, results showed that the time of progression to a more sever stage, was slower among those treated with vitamin D [15].

Only few studies are available that have investigated vitamin D status among older adults with MCI or dementia and they indicate a higher prevalence of vitamin D deficiency compared to cognitively intact individuals [11, 16]. However, studies that examine the associations between lifestyle and vitamin D status in subjects with MCI or dementia are not available.

Given these considerations, we investigated 25OHD in different cognitive status groups among old adults from the Age Gene/Environment Susceptibility-Reykjavik Study (AGES-RS). The aims were to examine (1) vitamin D status among old adults with dementia, MCI and normal cognitive function, (2) whether participants with dementia and MCI have poorer lifestyle than cognitively intact individuals, (3) the associations between lifestyle and 25OHD in each cognitive status group using stratified, linear regression analysis.

Methods

Study population

The current cross-sectional analysis is based on data from the AGES-RS, which in general examined risk factors for diseases and disability in old age that includes environmental factors, genetic susceptibility, and their interactions. The AGES–RS is a continuation of the Reykjavik Study from the Icelandic Heart Associations (IHA). The Reykjavik Study was initiated in 1967 and included men and women born in the period 1907–1935 living in the Reykjavik area [17]. During 2002–2006, 5764 persons were chosen randomly from the survivors of the Reykjavik Study cohort and re-examined for the AGES–RS. Participants underwent a clinical examination, completed questionnaires, and completed a cognitive test battery. Details on the study design and the baseline AGES-RS assessments have been given elsewhere [18, 19]. In the current study, we used data from AGES-RS obtained between 2002 and 2006.

Mild cognitive impairment and dementia

Assessment of cognitive function was done following a three-step protocol to identify subjects with dementia or MCI. First, the Digit Symbol Substitution Test [20] and the Mini-Mental State Examination [21] were administered to the total sample. Participants who scored 23 or lower on the Mini-Mental State Examination or had a raw score of 17 or lower on the Digit Symbol Substitution Test were administered a second diagnostic cognitive test battery. Participants who scored 8 or more on Trails B [22] that was the ratio of time taken for "Trails B/Trails A" (corrected for the number correct: [{time Trails B/number correct Trails B}/{time Trails A/number correct Trails A}]) or had lower than total score of 19 for the four immediate recall trials of the Rey Auditory Verbal Learning [23] went on to a third step. This step included a neurological examination and a proxy interview regarding medical history, social, cognitive, and daily functioning changes of the participant. Based on the three-step protocol, an assessment of dementia was made by a team composed of a geriatrician, neuroradiologist, neurologist, and neuropsychologist a according to international guidelines from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [24].

The assessment for MCI was also made by the team, the criterion for having MCI was having degeneration in either memory or in one other cognitive domain, or having deficits in at least two cognitive domains without sufficiently severe

cognitive function impairment or loss of activities of daily living to constitute dementia [25].

For the statistical analyses, participants were categorized into three groups: demented, MCI, and cognitively normal.

Serum 25-hydroxy-vitamin D measurement

The accredited laboratory from the Icelandic Heart Association conducted 25OHD measurements using the Liaison chemiluminescence immunoassay (DiaSorin Inc., Stillwater, Minnesota). The inter-assay coefficient of variation was < 6.5% when calculated data are from measurements using a frozen serum pool as the control sample and < 12.7% when calculated data is from measurements using liaison quality controls. Existing serum 25OHD levels were then standardized according to the international Vitamin D Standardization Program (VDSP) as previously described [19, 26]. For the analyses, standardized 25OHD were categorized into three groups based on Guidelines for Health Professionals from the National Institutes of Health (2014): deficient (≤ 30 nmol/L), insufficient (31–49 nmol/L), normal–high levels (≥ 50 nmol/L).

Covariates

The following covariates were assessed and used for statistical analysis: education (primary vs. at least secondary), BMI (both as kg/m^2 and categorical as underweight (< 20 kg/m^2), normal weight (20 to < 25 kg/m^2), overweight (25 to < 30 kg/m^2), and obese (≥ 30 kg/m^2), current smoking (yes vs. no), current alcohol consumption (yes vs. no), cod liver oil (daily vs. not daily), vitamin D supplements (yes vs. no), fatty fish consumption (< 3 vs. ≥ 3 times/week), leisure time physical activity (PA) (≤ 3 h vs. > 3 h), and medication (≤ 4 vs. ≥ 5).

Type 2 diabetes mellitus (yes vs. no) was defined by a physician's diagnosis of diabetes, use of diabetes medication and/or fasting blood glucose of > 7.0 mmol/L. Hypertension (yes vs. no) was defined by a physician's diagnosis of hypertension, use of hypertension medications and/or blood pressure above 140/90 mm Hg.

Analytical sample

Of the total cohort ($N = 5764$), 5519 had measurement of 25OHD, and 5512 of those had data with complete assessment of cognitive status. Participants were categorized into three groups according to cognitive status. After excluding 350 participants with missing values on covariates, the number in each cognitive status group was 4363 (83.8%) with normal cognitive status, 492 (9.5%) with mild cognitive impairment and 307 (5.9%) with a dementia diagnosis after data cleansing. Therefore, the final analytical sample included 5162 participants.

Statistical analysis

Statistical analyses were carried out using IBM SPSS version 24.0 (SPSS, Chicago, IL, USA).

Baseline data

Differences in demographic and health characteristics among the three cognitive status groups were calculated using Chi-square test for categorical variables (with Bonferroni correction) and analysis of variance for continuous variables (with LSD post hoc test), crude and age adjusted. The level of statistical significance was set at $P < 0.05$ ($P < 0.016$ for the Bonferroni and LSD correction).

Linear regression models

Liner regression models (LEM) were used to address the research questions proposed initially by applying the continuous outcome of serum 25OHD levels as dependent variable throughout all calculations.

First, the differences in serum 25OHD levels were calculated among the cognitive status groups (all cognitive status groups in one model) using the normal cognitive status group as a referent group. Second, the associations between lifestyle and serum 25OHD levels were calculated using stratified analysis; therefore, calculating the associations separately in each cognitive status group.

The level of statistical significance was set at $P < 0.05$.

The differences in 25OHD levels among cognitive status groups—unstratified analysis

The differences in serum 25OHD levels among the three cognitive status groups were examined, by calculating the changes in beta between dementia group and MCI group as compared to the normal cognitive status group. The differences were calculated in two linear regression models (general linear model-univariate in SPSS): model 1: crude; model 2: additionally included age, gender, season, and education.

The associations between lifestyle and 25OHD-stratified analyses by cognitive status

The initially proposed research question, regarding associations between lifestyle and vitamin D levels, was examined through linear regression models (general linear model-univariate in SPSS), analyses were done separately for all three cognitive status groups (dependent variable: serum 25OHD).

Statistical correction for age, gender, season, and medication were applied.

Results

As shown in Table 1, most of the baseline characteristics were significantly different between the three groups and in general, the health characteristics of participants with dementia and MCI were worse than among cognitively intact participants. Both, the lowest mean 25OHD and highest prevalence of vitamin D deficiency were observed among dementia participants; however, the mean 25OHD of this group was still within the normal range (Table 1). Physical activity levels were low among the dementia group (63% reported no activity); however, the use of supplements was proportionally the highest among the same group (36%).

Table 2 shows the differences in 25OHD between the three groups using linear models accounting for potential confounders. Subjects with dementia had lower 25OHD levels by around 3.7 nmol/L when compared to subjects with normal cognitive function and neither lifestyle nor medicine use explained this difference (results not shown in table). MCI subjects had lower 25OHD levels in the crude analysis; however, this difference disappeared after statistical correction.

Table 3 shows the associations between lifestyle and 25OHD using linear models stratified by cognitive status. Among subjects with dementia and MCI, cod liver oil intake and vitamin D supplements were the only lifestyle variables significantly associated with 25OHD. Those two variables showed the strongest results in heightening 25OHD levels, ranging from 7.12 to 9.18 nmol/L (cod liver oil-daily intake) and 4.41–11.52 nmol/L (supplement use).

Table 1 Demographic and health characteristics according to cognitive status among the participants (shown as mean ± SD or as %)

	Dementia ($n=307$)	Mild cognitive impairment ($n=492$)	Normal cognition ($n=4363$)	*P* value	*P* value[a]
Age (years)	81.7 ± 5.5*	80.4 ± 5.7*	75.8 ± 5.2	< 0.001	
Female	54%*	50%*	59%	< 0.001	< 0.001
Education (primary)	34%*	41%*	19%	< 0.001	< 0.001
Smoking (yes)	9%	10%	9%	0.51	0.012
Alcohol consumption (yes)	48%*	52%*	66%	< 0.001	< 0.001
25OHD (nmol/L)	53.8 ± 19.6*	55.8 ± 19.0*	57.6 ± 17.7	< 0.001	< 0.001
Deficient (≤ 30 nmol/L)	16%*	12%*	7%	< 0.001	
Insufficient (30–49 nmol/L)	24%	27%	25%		
Normal (≥ 50 nmol/L)	60%	61%	68%		
Weekly physical activity				< 0.001	< 0.001
No activity	63%*	61%*	44%		
≤ 3 h	29%	30%	40%		
> 3 h	8%	9%	16%		
BMI (kg/m^2)	26.3 ± 4.3*	26.8 ± 4.4	27.1 ± 4.4	0.01	0.86
Underweight	2%*	2%	1%		
Normal weight	38%	35%	31%		
Overweight	43%	41%	45%		
Obese	17%	22%	23%		
Cod liver oil (yes)	34%*	40%	42%	0.04	0.28
Vitamin D suppl. (yes)	36%*	31%	30%	< 0.01	< 0.001
Fatty fish (≥ 3*x*/week)	11%	12%	14%	0.82	0.09
Hypertension[b]	83%	85%*	80%	0.01	0.48
Type 2 diabetes[c]	18%*	14%*	11%	< 0.001	< 0.001
Medicine count ≥ 5	58%*	46%*	38%	< 0.001	< 0.001

25OHD 25 hydroxy-vitamin D, *BMI* body mass index

*Significantly different from the normal cognitive function group

[a]Age-adjusted P value

[b]Hypertension, those with systolic BP over 140 mmHg, diastolic BP > 90 mmHg or on hypertensive medication

[c]Diabetes mellitus was defined by physician's diagnosis of diabetes or use of diabetes medication

Table 2 Differences* in 25-hydroxy-vitamin D levels among dementia and mild cognitive-impaired participants using liner regression

Dependent variable: 25OHD	Model 1				Model 2			
	β	95%CI		*P* value	*β*	95%CI		*P* value
Dementia (*n* = 307)	− 3.733	− 5.748	− 1.717	< 0.01	− 3.880	− 5.968	− 1.791	< 0.01
MCI (*n* = 492)	− 1.785	− 3.429	− 0.141	0.033	− 1.483	− 3.196	0.230	0.090
Normal Cognition** (*n* = 4346)								
Age (years)					0.085	− 0.006	0.176	0.067
Male[a]					4.077	3.081	5.074	< 0.01
Season (summer)[b]					2.824	1.353	4.295	< 0.01
Education (primary)[c]					− 4.284	− 6.096	− 2.472	< 0.01

β β Coefficient; model 1: crude. model 2: corrected for age, sex, season, and education

*Based on univariate general linear model

**Reference group

Compared to [a]female, [b]winter, [c]university degree

Table 3 Associations* between lifestyle and 25-hydroxy-vitamin D levels** in the three groups of cognitive status

Dependent variable: 25OHD	Dementia (*n* = 307)				Mild cognitive impairment (*n* = 492)				Normal cognition (*n* = 4363)			
	β	95%CI		*P* value	*β*	95%CI		*P* value	*β*	95%CI		*P* value
Physical activity (≥ 3 h/week)	1.77	3.078	6.626	0.47	3.38	− 0.45	6.95	0.07	2.82	1.73	3.79	< 0.01
BMI (< 30 kg/m^2)	2.68	− 3.134	8.384	0.36	0.99	− 2.73	5.32	0.63	5.24	4.43	6.80	< 0.01
Cod liver oil (daily)	7.12	2.908	12.582	< 0.01	8.91	5.25	12.79	< 0.01	9.18	7.99	10.37	< 0.01
Supplements (yes)	11.52	6.825	15.810	< 0.01	6.37	3.82	11.01	< 0.01	4.41	3.32	5.49	< 0.01
Smoking (no)	0.59	− 6.21	7.41	0.86	4.31	− 1.17	9.78	0.21	4.79	3.26	6.69	< 0.01
Alcohol (yes)	2.11	− 6.13	2.73	0.32	1.25	− 2.07	4.58	0.79	2.67	1.59	3.74	< 0.01
Fatty fish (≥ 3*x*/week)	2.35	3.86	8.56	0.11	4.55	− 0.41	9.52	0.07	2.63	1.21	4.10	< 0.01

The statistical model additionally includes age, gender, season, and medication

β β Coefficient, *BMI* body mass index

*Based on univariate general linear model

**25OHD as the dependent variable

Among participants with normal cognitive function, all the investigated lifestyle variables were significantly associated with 25OHD.

Among subjects with dementia, there was similar trend for positive associations between physical activity and 25OHD levels as seen among the normal cognitive function subjects, even though results did not reach statistical significance. The following three variables were associated with the highest 25OHD levels among normal cognitive function subjects; daily use of cod liver oil, body mass index (< 30 kg/m^2), and no smoking.

Tables 2 and 3 was further recalculated with random sampling equalizing the three cognitive function groups matching the smallest group (*N* = 307). Random sampling changed the results only marginally and the new calculations were largely in agreement with previous analyses (results not shown in table).

Discussion

The current large cross-sectional study investigated the associations between lifestyle and 25OHD in community-dwelling old adults with dementia, MCI and normal cognitive function According to age-adjusted analysis, participants with dementia and MCI had significantly lower 25OHD and they had poorer lifestyle than participants with normal cognitive status, although the differences were small and not clinically relevant. Lifestyle was differentially associated with 25OHD in all three cognitive status groups, whereas the strongest associations were observed among the normal cognitive status group.

25OHD and cognitive status

In agreement with previous studies [10], we found lower 25OHD in participants with MCI and dementia. After statistical correction for lifestyle variables, the differences in 25OHD between MCI and normal group disappeared but remain significant in the dementia group.

It is possible that the lower vitamin D levels in participants with dementia are disease related [27] or a result of residual confounding, even though we controlled extensively for confounding factors. In general, lifestyle was related to 25OHD; however, there were some differences observed between the three cognitive status groups. Vitamin D supplements were associated with higher serum vitamin D levels in all three groups and we saw that they were used more frequently in participants with dementia than in the other two groups. Also, daily consumption of cod liver oil was positively associated with vitamin D levels in all three groups; however, the most frequent consumption was seen in the normal cognitive status group. High consumption of fatty fish was positively associated with 25OHD in the normal cognitive group only. The results indicate that individuals with dementia rely more on the use of supplements than cognitively intact individuals, possibly leading to sufficient 25OHD levels, thus explaining the small differences in 25OHD observed between the three groups.

Lifestyle and 25OHD levels among the cognitive status groups

As reported previously [28], we found that participants with dementia were less physically active. Studies suggest that individuals with dementia benefit from physical activity measured by overall health and well-being [8]. A study by van der Roest et al. revealed that in 40% of people with dementia, the needs for physical activity were not fulfilled [29]. In our study, majority of participants with dementia and MCI were physically inactive, 63% and 61%, respectively, which was a higher prevalence than in the group of people with normal cognitive function. This low physical activity among participants with dementia is alarming, since studies have shown that physical inactivity in individuals with dementia is associated with increased risk of cardiovascular disease, metabolic aberrations, and an accelerating progression of dementia [30, 31]. In the final analysis, there was a lack of a significant associations between physical activity and 25OHD in the dementia and the MCI group, this might be explained by several factors: a high proportion of participants with dementia and MCI was inactive, and the small proportion of active participants might have been more likely to participate in a supervised indoor activity without sufficient amount of sunlight exposure.

Mean levels of BMI were not significantly different between the groups in the age-adjusted analysis. When examining the BMI levels by group, participants with dementia had a somewhat higher proportion of underweight and normal weight compared to the group with normal cognitive status. Although, differences in the BMI levels were small, equaling around 3 kg body weight, they might be of importance, since studies have suggested that older adults who are overweight compared to those who are normal weight show better cognitive performance [32] and weight loss may be a preclinical indicator of Alzheimer disease [33]. Further, weight loss is associated with a faster progression of dementia and with nursing home placement [34]. As previously observed [35], BMI was negatively associated with 25OHD, but we could observe this only in the normal cognitive status group and no significant relation between BMI and 25OHD was observed in the dementia or MCI groups.

In our study, smoking prevalence was low in all three groups. In the group with normal cognitive function, it was related to lower 25OHD by around 4 nmol/L which has been seen before [36]. A negative correlation between 25OHD levels and smoking could possibly be explained by the fact that smoking is usually accompanied by a less healthy lifestyle. However, in our analyses, the difference remained although we corrected for various lifestyle factors.

Participants with normal cognitive function reported more frequent alcohol consumption than the other two groups. For this group, we also observed a positive association between alcohol consumption and 25OHD. Similar results were reported previously in a Finish cross-sectional study [37]. However, a recently published review article on vitamin D and alcohol consumption found mixed results, indicating that the direction of the association between vitamin D and alcohol depends very much on the investigated population, e.g., alcoholic patients vs. moderate drinkers [38]. Newer experimental evidence actually does not support neither a positive nor a negative causal effect of moderated drinking on 25OHD [39].

Strength and limitations

The current study was of cross-sectional design which cannot differentiate cause and consequence of an observed association between lifestyle and vitamin D. The MCI ($N=492$) and dementia ($N=307$) groups had smaller statistical power associated with the total number of participants; therefore, a random sampling was applied equalizing the group size across cognitive function groups, and all analyses were recalculated. This procedure did not change the results significantly.

To our best knowledge, there are currently no studies available that compare serum vitamin D levels among

different cognitive status groups and further examine the associations between different lifestyle factors and serum vitamin D levels. It is a strength of this study, that it comprised a variety of health-related, socioeconomic, and lifestyle variables so we could adjust for number of important confounders in the statistical analysis.

Conclusion

Community-dwelling adults with dementia in Iceland are at higher risk for vitamin D deficiency when compared to healthy individuals, although the majority has still vitamin D levels within the normal range. Older people with dementia seem to rely more on vitamin D supplements than their healthy counterparts. Physical activity reported among participants with dementia and MCI is low and is not associated with 25OHD levels in these groups. Although participants with dementia and MCI had poorer lifestyle than healthy participants, differences in lifestyle did not fully explain the observed lower levels of 25OHD in the dementia group.

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Data availability Data are not available due to laws of the Icelandic Data Protection Authority.

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Statement of human and animal rights The study was approved by the National Bioethics Committee in Iceland (approval VSN-00-063), the Data Protection Authority, and by the National Institute on Aging Intramural Institutional Review Board.

Informed consent Written informed consent was obtained from all participants.

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Paper III

Title: Body weight changes and longitudinal associations with cognitive decline among community dwelling old adults.

Abstract

INTRODUCTION: We aim to investigate the longitudinal associations between changes in body weight (BW) and declines in cognitive function and risk of MCI/dementia among cognitively normal individuals 65 years or older

METHODS: Data from Age-Gene/Environment-Susceptibility-Reykjavik-Study on 2620, were examined using multiple logistic regression models. Cognitive function included speed of processing (SP), executive function (EF) and memory function (MF). Changes in BW were classified as; weight loss (WL), weight gain (WG) and stable weight (SW).

RESULTS: With a mean follow-up of 5.2 years, 843 participants (32.2%) lost weight, 505 (19.3%) gained weight, and 1272 (48.5%) were weight stable. Participants who experienced WL were significantly more likely to have declines in MF and SP compared to the SW group. Weight changes were not associated with EF. Weight loss was associated with a higher risk of MCI, while WG was associated with a higher dementia risk, when compared to SW.
DISCUSSION: Significant BW changes in older adulthood may, independently, indicate impending changes in cognitive function.

Keywords: cognitive function, speed of processing, memory function, executive function, mild cognitive impairment, dementia, body weight changes, nutrition, APOE $\varepsilon 4$.

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1. INTRODUCTION

The increase of aging populations around the world comes with a burden of neuropsychological disorders including mild cognitive impairment and dementia, which are multi-factorial disorders that are determined by an interplay of environmental factors and genetic susceptibility [1]. Older age remains the strongest risk factor for dementia although many modifiable risk factors have been suggested by observational studies [2] being estimated to account for at least 30 percent of dementia occurrence and these risk factors include a variety of life-style related factors, e.g., physical activity (PA), body mass index (BMI) and nutrition [1-6].

Although prior studies have associated BMI with cognitive function and dementia results have been conflicting due to, variability in study design [7] and whether BMI is measured in mid-life or late-life [8, 9]. Some studies showed that high mid-life BMI is associated with the risk of developing dementia [9], but conversely, the same is true for low BMI when measured at late life [8]. However, according to a recent meta-analysis current available evidence does not support a clear association between overweight/obesity and incident dementia in old age [10].

Fewer studies are available on changes of body weight and cognitive function during old adulthood. It has been shown that weight loss is associated with the risk of dementia [11], it has also been suggested that weight loss is rather a consequence of the preclinical phase of dementia and this suggests a reverse causation between weight loss and dementia. However, associations between cognitive function and weight loss as well as weight gain are less clear.

The mechanism relating weight loss to cognition are not fully understood but recent studies have suggested that apathy, anxiety, depression and irritability among dementia and MCI

cases affect appetite [12]. However, it is also conceivable that weight loss could accelerate brain atrophy before the onset of MCI or dementia [11].

Weight loss coming from an inadequate dietary intake, eventually, leads to deficiency in critical nutrients [13] making nutrition important in these associations. Another consideration is whether body weight changes are associated with cognition via known or suggested risk factors for dementia, e.g., vitamin D has been suggested to be associated with cognitive decline [14] as well as APOE ε4 [15, 16]

In order to gain more knowledge on the relation between cognitive function and body weight, we conducted this analysis based on data from the longitudinal Age Gene/Environment Susceptibility-Reykjavik Study (AGES-RS). The aim of this study was to 1) investigate the longitudinal associations between changes in late life body weight and declines in cognitive function and risk of MCI/dementia in community dwelling old adults with normal cognitive function at baseline. Further adding to the novelty of this study, we considered potential confounding of physical activity, nutritional factors and APOE ε4 when examining the associations between changes in body weight and cognitive function.

2. METHODS

2.1 Study population and study design

The current longitudinal analysis is based on data from the AGES-Reykjavik study (N= 5718), which examined risk factors for diseases in old age, including environmental factors, genetic susceptibility and their interactions. Briefly, the AGES–Reykjavik (AGES I) study was enrolled in 2002-2006 as a continuation of the population based Reykjavik Study (RS) in Iceland, initiated in 1967, including men and women born in 1907–1935 and living in the Reykjavik area [17]. Detailed baseline information have been described in the AGES-study paper [18]. Between 2007-2011, all surviving AGES I participants (58%, N=3316) returned

for a 5-year follow-up visit (AGES II). The current study included participants who were cognitively normal at baseline and had relevant follow-up examination including cognitive tests and body mass index. The study was approved by the National Bioethics Committee in Iceland (approval VSN-00-063), the Data Protection Authority, and by the National Institute on Aging Intramural Institutional Review Board. Written informed consent was obtained from all participants.

2.2 Anthropometrics

Weight and height were measured and BMI was calculated as kg/m^2. Participants were categorized as underweight (baseline BMI less than 18.5), normal weight (BMI 18.5–24.9), overweight (BMI 25.0–29.9) and obese (BMI ≥ 30.0). Participants were further categorized into weight stable, weight gain and weight loss if they had lost or gained ≥ 3 kg during follow-up as is considered clinically relevant weight changes [19, 20].

2.3 Cognitive function assessment

Assessment of cognitive function included seven tests, both at baseline and follow-up, focusing on three cognitive domains, i.e., memory, processing speed and executive function. For each of the domains, a composite score was constructed based on a theoretical grouping of the tests and by converting raw scores into standardized z scores reflecting the distribution within the study sample as previously described [21]. The inter-rater reliability for all tests was excellent (Spearman correlation coefficients range 0.96–0.99) (22). The memory composite measure included the immediate and delayed-recall portions of a modified version of the California Verbal Learning Test (23). The processing speed composite measure included the Digit Symbol Substitution Test (24), the Figure Comparison Test (25) and the Stroop Test (26) Part I (reading) and Part II (color naming). The executive function composite

measure included the Digits Backward Test (24) and the Stroop Test, Part III (word-color interference). The three domains of memory, processing speed and executive function composite measures were each used as a continuous variables.

2.4 MCI and Dementia

Assessment of cognitive function was done following a three-step protocol to identify subjects with dementia or MCI. First, the Digit Symbol Substitution test [22] and the Mini-Mental State Examination [23] were administered to the total sample. Participants who scored 23 or lower on the Mini-Mental State Examination or had a raw score of 17 or lower on the Digit Symbol Substitution test were administered a second diagnostic cognitive test battery. Participants who scored 8 or more on Trails B [24] which was the ratio of time taken for "Trails B/Trails A" or had lower than total score of 19 for the four immediate recall trials of the Rey Auditory Verbal Learning [25] went on to a third step. This step included a neurological test and a proxy interview regarding medical history, social, cognitive, and daily functioning changes of the participant.

A consensus diagnosis of dementia made by a team composed of a geriatrician, neurologist, neuropsychologist, and a neuroradiologist was made according to international guidelines from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (28).

2.5 Covariates

2.5.1 Baseline demographic data

Participants were asked about their age, gender, current marital status categorized as married, widowed, divorced or single. Education was categorized into four levels (elementary school, high school, undergraduate, more than undergraduate education.

2.5.2 Blood pressure

Blood pressure (mmHg), was measured in a recumbent position using mercury sphygmomanometer and a large cuff on the right arm (with a few exceptions) after participants had rested for 5 minutes.

2.5.3 Lifestyle and nutritional data

The accredited IHA laboratory performed 25OHD measurements in batch using unfrozen serum samples and the Liaison chemiluminescence immunoassay (DiaSorin Inc, Stillwater, Minnesota). Existing serum 25OHD levels were then standardized according to the international Vitamin D Standardization Program as previously described (20).

Leisure time physical activity was assessed by a self-reported questionnaire and categorized into 1) none, 2) ≤ 3 hours/week or 3) > 3 hours/week. Smoking status was evaluated as ever vs. never smoker. Alcohol consumption was evaluated as either currently consuming vs. not consuming.

2.5.4 Medication use and APOE ε4 genotype

Participants were instructed in advance to bring all medication they had used during the preceding two weeks before the clinic visit and were categorized into ≤ 4 medication vs. ≥ 5 medication. APOE ε4 alleles were genotyped on a subsample of 2113 people using standard methods [26]. Basic characteristics of this subsample did not differ from the remaining sample. Participants were considered APOE ε4 positive if they carried ε3/4, and ε4/4 genotype otherwise if they carried ε2/2, ε2/3 and ε3/3 they were considered APOE ε4 non-carriers.

2.6 Analytical Sample

From the original sample size of 5764 in the provided data base, 3316 participants completed the follow-up measurements. Participants with an MCI (n = 204) or dementia diagnosis (n = 47) at baseline and participants having incomplete data (n=445) were excluded from the present analysis. From the remaining sample, 2620 participants had a complete data set of relevant variables and were thus included into the present study.

2.7 Statistical analysis

Statistical analyses were carried out using IBM SPSS version 22.0 (SPSS, Chicago, IL, USA). Demographic, anthropometric, lifestyle- and nutritional data, medication use and APOE $\varepsilon 4$ genotype variables were used to describe baseline characteristics of the participants (Table 1) according to body weight change categories. We used chi-square test for categorical variables and ANOVA for continuous variables to test for statistical differences.

In order to calculate longitudinal associations between changes in body weight and the three domains of cognitive function (Tables 2-4), univariate general linear models (GLM) were applied controlling for various confounders. For each outcome variable the following 3-step model was applied: Model 1 adjusted for age, gender and baseline cognitive function. Model 2 additionally adjusted for 25OHD, baseline BMI and PA. Model 3 additionally adjusted for marital status, smoking, education, APOE $\varepsilon 4$ and medication use.

In order to calculate whether changes in body weight predict onset of MCI or dementia (Tables 5 and 6), regression analyses were applied controlling for various confounders as outlined for GLM above.

The level of statistical significance was set at $p < 0.05$.

3. RESULTS

3.1 Baseline

Baseline characteristics of the participants categorized by weight change during follow-up can be seen in Table 1. Most of the baseline characteristics among participants were significantly different between the 3 categories. Participants in the weight loss group had slightly higher baseline BMI, lower vitamin D levels, fewer were married, and lower proportion of physical activity. Participants who were in the weight loss category displayed the lowest z-scores in all three cognitive function domains.

3.2 Follow-up

During a mean follow-up of 5.2 years, 843 participants (32.2%) lost weight (-6.7 ± 3.8 kg), 505 (19.3%) gained weight (5.7 ± 2.9 kg) and 1272 (48.5%) were weight stable (-0.1 ± 1.5 kg). In these categories 83 (9.8%), 33 (6.5%) and 70 (5.5%) participants, respectively, were diagnosed with MCI and 27 (3.2%), 26 (5.1%), 26 (2.0%) participants, respectively, were diagnosed with dementia. Baseline BMI categories (underweight, normal weight, overweight, obese) were not related to cognitive function or MCI/dementia at the end of follow-up.

Tables 2-4 show the longitudinal associations between weight change categories and cognitive function based on GLM. Weight loss was associated with a lower memory function and lower speed of processing after follow-up when compared to weight stable. As shown in models 1-3, correction for baseline cognitive function and BMI, demographic factors, lifestyle as well as medication and APOE $\varepsilon 4$ variables did only marginally change these results. However, weight loss was not associated with executive function.

Table 5 and 6 show that weight change categories were associated with the development of MCI and dementia during follow-up based on logistic regression. Weight loss was associated with a higher likelihood of MCI when compared to weight stable. Further, both weight loss

and weight gain were associated to a higher dementia risk when compared to weight stable. Similar to GLM results show above, correction for baseline BMI, demographic factors and lifestyle as well as medication and APOE ε4 variables did only marginally change these results.

Inclusion of APOE 4 ε4 and 25OHD as covariates did not change the results. Nutritional factors related to vitamin D levels, i.e. cod liver oil consumption and consumption of fatty fish did not have significant associations with any of the cognitive function domains (results not shown in table) and therefore did not alter the associations between body weight changes and cognitive function.

4. DISCUSSION

This large longitudinal study investigated the associations between body weight changes and cognitive function among community dwelling old adults who had normal cognitive function at baseline. We found that participants who lost weight during the follow-up period, had lower cognitive function after follow-up compared to weight stable or weight gaining participants. We also found that these participants had a higher risk of developing MCI. Further, our study suggests that participants who gained weight during follow-up were at an increased risk for dementia compared to weight stable participants. BMI categories themselves were neither related to cognitive function nor to risk of MCI or dementia.

Since the risk of reverse causation can distort the relationship between dementia and weight loss, the current study exclusively included participants with normal cognitive function at baseline which reduces this risk. The associations between body weight changes and cognitive function we found are in agreement with several previous studies on this topic [1-6]. When comparing results from different studies, it has to be considered that longitudinal

studies concerning body weight, BMI and cognitive function use various techniques measuring cognitive abilities with different endpoints ranging from mild cognitive impairment to dementia. Also, studies enroll participants of different age groups and it has been shown that, e.g., high BMI can be both detrimental as well as protective for a given health outcome depending on the participants' age [27-29].

In the present study, weight loss was associated with faster cognitive decline for memory and speed of processing when compared to weight stable or weight gaining participants. Interestingly, intentional weight loss in obese/overweight adults has been reported to be associated with improvements in performance across various cognitive domains [30]. However, in a recently published cohort study among community-dwelling elderly, weight loss predicted higher cognitive decline over a 5 year follow-up, independently of baseline BMI [31].

Further, it has been reported that both weight loss and weight gain were associated with poor cognitive performance in middle-aged and older women compared with women with stable weight after seven years of follow-up [32].

In our study weight loss during the study period was associated with 85% higher risk of MCI diagnosis. This is in agreement with a large prospective longitudinal cohort study from the USA in which weight loss was associated with a higher risk of incident MCI independent from BMI [33]. Similar results were reported in old adults from an African study with ten years of follow-up [34].

Contrary to our expectations, we found that weight gain during follow-up was associated with a greatly increased risk of dementia. In contrast, two cohort studies from the USA reported weight loss to be associated with a higher risk of incident dementia [31, 35] whereas weight gain did not have any significant associations [31]. No information is available in published

literature linking weight gain with dementia risk, although there are several studies published having linked obesity to dementia risk [36, 37].

However, according to a recent meta-analysis current available evidence does not support an association between overweight/obesity and incident dementia in old age [10]. There are many studies available which link BMI categories with cognitive function, MCI and dementia [29, 38, 39]. In the present study BMI categories were not related to cognitive decline or MCI or dementia diagnosis. It has to be considered that of our study population, actually very few were underweight (n = 22) which excludes the possibility of a meaningful statistical analysis. On the other hand, our study shows that body weight change is an important predictor of future cognitive function independent from BMI category.

There are several plausible explanations as to how body weight change can be associated with cognitive function. However, they fail to explain our findings entirely because body weight stability has been associated with an intact social environment and might in general reflect good health of an old adult. Weight loss on the other hand might be an early sign of deteriorating health. Although body fat is associated with increased levels of leptin [40] which might act as protective factor for cognition in old age [41], weight gain in old adulthood can also be associated with sedentary lifestyle and physical inactivity.

In fact, the distribution of body fat might be crucial to understand the inconclusive associations between of obesity and dementia [42]. In the present study, we cannot distinguish between visceral- and subcutaneous fat in the weight gain group, but previous studies have shown an association between visceral adipose tissue (rather than subcutaneous adipose tissue) and microstructural brain tissue damages as well as poorer brain connectivity [42, 43]. In this perspective it is appropriate to discuss a study by Spauwen et al. (2017), which also is a cross-sectional study using data from AGES-Reykjavik, showing that a higher amount of

subcutaneous fat was negatively associated with the risk of dementia at baseline [44]. Thus, visceral fat might be a driving force in these associations between weight gain and dementia. Previous studies have shown that type 2 diabetes increases the risk of dementia [45], we therefor considered type 2 diabetes in additional analyses. Our results showed that the association between weight changes and cognitive function/MCI/dementia was unchanged when controlling for type 2 diabetes.

Physical activity has been shown to have positive implications for various health-related outcomes among older adults, [46-48], including brain health [4, 49]. As shown in Table 1, the proportion of physical inactivity among the weight gain group was high or 38%. Additional calculations (not shown) stratifying by physical activity levels showed that the weight gain - dementia associations were mainly driven by participants who did not engage in any physical activity. This further confirms the protective effects of physical activity among this group of older adults.

In the present statistical analyses, we included several potential confounders. The extensive statistical correction only marginally changed results in the GLM and logistic regression models. Unexpectedly physical activity and nutritional factors were not significantly associated with any of the cognitive function domains or risk of dementia/MCI diagnosis in the final analysis and did therefore not confound the observed associations between body weight changes and cognitive function. Further, APOE ε4, although being significantly related to cognitive function in our study, did not change the observed associations between body weight change and cognitive outcomes.

5. STRENGTHS, LIMITATIONS AND FUTURE DIRECTIONS

It is a strength of our longitudinal study that it included a large number of participants who underwent detailed examinations at baseline and at follow-up of the study. A considerable

number of covariates has been used in the present analysis in order to investigate whether physical activity, nutrition, APOE ε4 or other demographic factors explain the relationship between body weight change and cognitive function. However, as our study design was of observational nature, we cannot exclude the possibility of bias and confounding effects on the observed associations between body weight change and cognitive function. Future intervention studies should address the question whether keeping body weight stable during old adulthood helps to maintain cognitive function and decreases risk of MCI and dementia. Also, since dementia is a hyper-term, representing a broad array of brain diseases we could not distinguish between common subgroups like Alzheimer´s disease and vascular dementia limiting precise interpretation of weight changes among older adults.

6. CONCLUSION

Our study showed that participants, who lost body weight during the follow-up period, had lower cognitive function after follow-up compared to weight stable or weight gaining participants and consequently these participants had a higher risk of developing MCI. In contrast to our expectations, we found that participants who gained weight during follow-up were at an increased risk for dementia compared to weight stable participants. Level of BMI categories themselves were neither related to cognitive function nor to risk of MCI or dementia. We conclude that keeping body weight stable during old adulthood is the best option to maintain cognitive function in old age.

Conflict of interest: The authors declare no conflict of interest.

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Table 1: Demographic and health characteristics according to weight groups among AGES-Reykjavik participants (N = 2620).

	weight loss (n=843)			weight gain (n=505)			no weight change (n=1272)			
	Mean	**±**	**SD**	**mean**	**±**	**SD**	**mean**	**±**	**SD**	**P-value***
Demographic data										
age (years)	75.4	±	4.8	74	±	4.5	74.5	±	4.7	<0.001
male (%)		38			39			44		0.01
female (%)		62			61			56		
education-basic (%)		31.3			23.5			45.2		0.032
married (%)		62.4			61.7			67.6		0.025
Lifestyle data										
physical inactivity (%)		42.0			38.4			35.9		0.008
alcohol-no (%)		32.1			35.5			29.2		0.055
smoke-yes (%)		8.3			10.1			7.8		0.213
Anthropometric data										
BMI (kg/m2)	28.1	±	4.4	27.5	±	4.5	26.6	±	3.9	<0.001
body fat (%)	30	±	7.4	29.6	±	7.7	28.2	±	7.8	<0.001
SBP (mmHg)	143	±	20	140	±	20	141	±	19	0.014
DBP (mmHg)	73.5	±	9.4	75	±	9	74.4	±	10	0.022
Laboratory data										
25OHD (nmol/L)	56.6	±	17	57	±	18.7	59.9	±	16.9	<0.001
Neuropsychological data										
memory (z-score)	0.032	±	0.885	0.173	±	0.890	0.154	±	0.867	0.001
executive (z-score)	0.030	±	0.730	0.060	±	0.762	0.131	±	0.729	0.004
speed (z-score)	0.104	±	0.687	0.061	±	0.705	0.163	±	0.678	0.007
Medication/APOE										
APOE 4 allele carriers (%)		1.9			1.8			1.4		0.589
Medication >5 (number)		35.5			34.4			31.5		0.100

*Chi-square test for categorical variables and ANOVA for continuous variables were to test for statistical differences.

Table 2: Associations* between weight change categories and memory function among AGES-Reykjavik participants (N = 2620)**.

	Model 1				Model 2				Model 3			
Parameter	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
Intercept	2.241	1.805	2.678	<0.001	2.177	1.673	2.682	0	2.15	1.561	2.738	<0.001
weight loss†	**-0.097**	**-0.156**	**-0.039**	**0.001**	**-0.097**	**-0.157**	**-0.037**	**0.001**	**-0.098**	**-0.157**	**-0.038**	**0.001**
weight gain†	**-0.015**	**-0.085**	**0.054**	**0.666**	**-0.014**	**-0.084**	**0.056**	**0.695**	**-0.009**	**-0.079**	**0.061**	**0.793**
baseline of dependent variable	0.71	0.678	0.741	<0.001	0.71	0.678	0.742	0.000	0.705	0.672	0.737	0.000
male§	-0.153	-0.207	-0.098	<0.001	-0.154	-0.209	-0.099	0.000	-0.176	-0.236	-0.117	<0.001
age (years)	-0.032	-0.037	-0.026	<0.001	-0.032	-0.038	-0.026	0.000	-0.032	-0.038	-0.026	<0.001
25OHD (nmol/L)					0.001	-0.001	0.002	0.276	0.001	-0.001	0.002	0.339
BMI (kg/m2)					0.001	-0.006	0.007	0.785	0.001	-0.006	0.007	0.838
physical activity = none#					0.024	-0.052	0.100	0.529	0.036	-0.041	0.113	0.357
physical activity = <3 h/week3					0.003	-0.069	0.075	0.935	0.003	-0.069	0.076	0.926
married‡									0.049	-0.067	0.166	0.408
widow‡									0.038	-0.086	0.162	0.547
divorced‡									-0.038	-0.192	0.117	0.633
smoking no##									0.05	-0.046	0.146	0.308
education basic§§									-0.082	-0.181	0.018	0.107
education lower§§									-0.067	-0.15	0.016	0.114
education higher§§									-0.085	-0.181	0.012	0.086
APOE_4									-0.007	-0.212	0.198	0.947
Medication use < 5¶									0.027	-0.028	0.083	0.337

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use. † compared to weight stable; § compared to female; # compared to PA>3h/week; ‡compared to single; ## compared to smoking yes; §§compared to university; ¶ compared to medication use ≥ 5;

Table 3: Associations between weight change categories and speed of processing among AGES-Reykjavík participants (N = 2620)*.

	Model 1				Model 2				Model 3			
Parameter	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
Intercept	1.396	1.038	1.754	<0.001	1.442	1.028	1.856	<0.001	1.293	0.813	1.773	<0.001
weight loss†	**-0.099**	**-0.147**	**-0.052**	**<0.001**	**-0.092**	**-0.140**	**-0.044**	**<0.001**	**-0.092**	**-0.140**	**-0.044**	**<0.001**
weight gain†	**-0.038**	**-0.093**	**0.018**	**0.189**	**-0.033**	**-0.089**	**0.023**	**0.245**	**-0.031**	**-0.088**	**0.025**	**0.276**
baseline of dependent variable	0.945	0.913	0.977	<0.001	0.941	0.908	0.973	<0.001	0.932	0.897	0.967	<0.001
male§	-0.081	-0.123	-0.038	<0.001	-0.088	-0.131	-0.045	<0.001	-0.101	-0.147	-0.054	<0.001
age (years)	-0.022	-0.026	-0.017	<0.001	-0.022	-0.027	-0.017	<0.001	-0.023	-0.028	-0.018	<0.001
25OHD (nmol/L)					0.001	-0.001	0.002	0.314	0.001	-0.001	0.002	0.412
BMI (kg/m2)					-0.002	-0.008	0.003	0.349	-0.003	-0.009	0.002	0.197
physical activity = none#					-0.032	-0.093	0.029	0.304	-0.030	-0.091	0.032	0.342
physical activity = <3 h/week[3]					0.000	-0.058	0.058	0.996	-0.003	-0.062	0.055	0.910
married‡									0.034	-0.060	0.128	0.473
widow‡									0.024	-0.075	0.124	0.630
divorced‡									-0.008	-0.132	0.116	0.900
smoking no##									0.069	-0.008	0.147	0.078
education basic§§									-0.034	-0.116	0.048	0.416
education lower§§									-0.037	-0.105	0.031	0.286
education higher§§									-0.023	-0.100	0.055	0.566
APOE_4									0.210	0.045	0.375	0.013
medication use < 5¶									-0.018	-0.062	0.027	0.438

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use. † compared to weight stable; § compared to female; # compared to PA>3h/week; ‡compared to single; ## compared to smoking yes; §§compared to university; ¶ compared to medication use ≥ 5;

Table 4: Associations between weight change categories and executive function among AGES-Reykjavík participants (N = 2620)*.

	Model 1				Model 2				Model 3			
Parameter	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
Intercept	0.832	0.421	1.242	<0.001	0.91	0.432	1.387	<0.001	1.157	0.602	1.712	<0.001
weight loss†	**-0.035**	**-0.091**	**0.021**	**0.224**	**-0.027**	**-0.084**	**0.031**	**0.362**	**-0.031**	**-0.088**	**0.026**	**0.285**
weight gain†	**-0.051**	**-0.118**	**0.015**	**0.128**	**-0.047**	**-0.113**	**0.02**	**0.168**	**-0.043**	**-0.11**	**0.024**	**0.205**
baseline of dependent variable	0.661	0.627	0.695	<0.001	0.658	0.624	0.692	<0.001	0.633	0.597	0.669	<0.001
male§	-0.103	-0.153	-0.053	<0.001	-0.109	-0.160	-0.058	<0.001	-0.138	-0.192	-0.084	<0.001
age (years)	-0.013	-0.019	-0.008	<0.001	-0.013	-0.019	-0.008	<0.001	-0.014	-0.020	-0.008	<0.001
25OHD (nmol/L)					0.000	-0.001	0.002	0.519	0.000	-0.001	0.002	0.705
BMI (kg/m2)					-0.003	-0.010	0.003	0.269	-0.003	-0.009	0.003	0.315
physical activity = none#					-0.024	-0.096	0.048	0.514	-0.003	-0.076	0.070	0.932
physical activity = <3 h/week[3]					0.006	-0.063	0.075	0.856	0.004	-0.065	0.073	0.912
married‡									-0.009	-0.120	0.102	0.872
widow‡									0.003	-0.114	0.121	0.956
divorced‡									-0.052	-0.199	0.095	0.490
smoking no##									0.082	-0.009	0.174	0.079
education basic§§									-0.220	-0.315	-0.124	<0.001
education lower§§									-0.138	-0.218	-0.058	0.001
education higher§§									-0.095	-0.186	-0.003	0.044
APOE_4									-0.126	-0.322	0.069	0.205
medication use < 5¶									0.019	-0.034	0.071	0.489

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use. † compared to weight stable; § compared to female; # compared to PA>3h/week; ‡compared to single; ## compared to smoking yes; §§compared to university; ¶ compared to medication use ≥ 5;

Table 5: Body weight change categories and risk of development of MCI among AGES-Reykjavik participants (N = 2620)*.

	Model 1				Model 2				Model 3			
Parameter	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
weight loss†	**1.855**	**1.322**	**2.603**	**<0.001**	**1.768**	**1.253**	**2.495**	**0.001**	**1.850**	**1.303**	**2.627**	**0.001**
weight gain†	**1.424**	**0.920**	**2.204**	**0.113**	**1.373**	**0.885**	**2.130**	**0.157**	**1.302**	**0.832**	**2.039**	**0.248**
male§	1.562	1.149	2.122	0.004	1.668	1.220	2.279	<0.001	2.292	1.626	3.231	<0.001
age (years)	1.144	1.108	1.181	<0.001	1.143	1.107	1.181	<0.001	1.139	1.099	1.181	<0.001
25OHD (nmol/L)					0.991	0.982	1.000	0.052	0.992	0.983	1.002	0.106
BMI (kg/m2)					1.007	0.969	1.046	0.722	0.999	0.960	1.040	0.977
physical activity = none#					1.259	0.782	2.026	0.344	1.073	0.661	1.742	0.775
physical activity = <3 h/week3					1.164	0.728	1.863	0.526	1.187	0.736	1.915	0.483
married‡									1.150	0.529	2.501	0.724
widow‡									1.352	0.610	2.994	0.458
divorced‡									1.643	0.630	4.284	0.310
smoking no##									0.725	0.414	1.271	0.262
education basic§§									8.480	3.711	19.378	<0.001
education lower§§									4.743	2.140	10.511	<0.001
education higher§§									1.921	0.767	4.815	0.164
APOE_4									0.764	0.225	2.595	0.666
medication use < 5¶									0.670	0.487	0.921	0.014

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use. † compared to weight stable; § compared to female; # compared to PA>3h/week; ‡compared to single; ## compared to smoking yes; §§compared to university; ¶ compared to medication use ≥ 5;

Table 6: Body weight change categories and risk of development of dementia among AGES-Reykjavik participants (N = 2620)*.

	Model 1				Model 2				Model 3			
Parameter	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
weight loss†	**1.463**	**0.838**	**2.552**	**0.181**	**1.426**	**0.812**	**2.507**	**0.217**	**1.517**	**0.858**	**2.684**	**0.152**
weight gain†	**3.031**	**1.720**	**5.341**	**<0.001**	**2.972**	**1.680**	**5.255**	**<0.001**	**3.071**	**1.724**	**5.469**	**<0.001**
male§	1.027	0.642	1.643	0.911	1.084	0.672	1.749	0.740	1.308	0.779	2.197	0.310
age (years)	1.182	1.129	1.239	<0.001	1.178	1.124	1.236	<0.001	1.182	1.123	1.245	<0.001
25OHD (nmol/L)					0.989	0.976	1.003	0.131	0.990	0.976	1.004	0.162
BMI (kg/m2)					0.988	0.934	1.046	0.686	0.984	0.928	1.044	0.588
physical activity = none#					1.054	0.539	2.062	0.878	0.948	0.479	1.878	0.878
physical activity = <3 h/week[3]					0.796	0.401	1.580	0.515	0.803	0.402	1.604	0.535
married‡									0.496	0.227	1.083	0.078
widow‡									0.364	0.157	0.844	0.018
divorced‡									0.294	0.075	1.158	0.080
smoking no##									0.742	0.319	1.726	0.488
education basic§§									5.263	1.748	15.849	0.003
education lower§§									2.422	0.828	7.083	0.106
education higher§§									2.449	0.779	7.701	0.125
APOE_4									0.589	0.130	2.670	0.492
medication use < 5¶									0.918	0.564	1.495	0.731

*Based on univariate GLM; **Excluded: participants with dementia and mild cognitive impaired at baseline; **Model 1**: age, gender and baseline cognitive function; **Model 2**: additionally 25OHD, body mass index and physical activity; **Model 3**: additionally marital status, smoking, education, apolipoprotein E and medication use. † compared to weight stable; § compared to female; # compared to PA>3h/week; ‡compared to single; ## compared to smoking yes; §§compared to university; ¶ compared to medication use ≥ 5;

Appendix

Supplementary table 1. Associations between the frequency of midlife physical activity (hrs/week) levels and 3 cognitive function domains* according to serum 25 hydroxyvitamin D concentrations among AGES-Reykjavik participants**(excluding dementia)

	Deficient (< 30nmol/L) n = 331		**Insufficient (31-49 nmol/L) n = 1058**		**Normal (≥50 nmol/L) n = 2915**	
	β	**p-value**	**β**	**p-value**	**β**	**p-value**
Memory Function	-0.009	0.72	-0.001	0.93	0.029	<0.001
Executive Function	-0.02	0.37	0.002	0.88	0.015	0.01
Speed of processing	-0.023	0.31	0.006	0.52	0.016	0.01

β= βeta coefficient

*continuous outcome

**Based on the general linear model (GLM)

Adjusted for: age, gender, education, body mass index, diabetes, hypertension, smoking, alcohol csumption, APOE ε4.

Supplementary table 2. Associations between lifestyle and 25-hydroxy-vitamin D levels in the three goups of cognitive status.

	Dementia (n=307)				Mild cognitive impairment (n=307)				Normal cognition (n=307)			
	β	95%CI		p-value	β	95%CI		p-value	β	95%CI		p-value
Physical activity (≥3h/week)	1.155	-3.362	5.672	0.61	4.337	-2.177	10.851	0.191	5.164	0.116	10.444	**0.05**
BMI (< 30 kg/m^2)	5.47	-0.401	11.349	0.68	1.041	-2.889	4.971	.603	6.902	3.206	10.597	**<0.01**
Cod liver oil (daily)	7.052	2.766	11.337	**<0.01**	3.926	0.462	7.391	**0.02**	6.921	3.829	10.012	**<0.01**
Supplements (yes)	6.058	1.602	10.515	**0.008**	10.036	6.349	13.724	**<0.01**	9.458	5.827	13.090	**<0.01**
Smoking (no)	2.668	-4.261	9.597	0.45	2.252	-3.188	7.692	0.41	4.79	.567	11.203	**0.03**
Alcohol (yes)	2.34	-3.29	7.97	0.41	-0.815	-5.356	3.727	0.72	4.171	0.142	8.201	**<0.01**
Fatty fish (≥3x/week)	1.426	-2.88	5.73	0.51	-1.755	-4.945	1.435	0.28	1.848	-1.157	4.852	**0.22**

Note: β = β Coefficient; BMI = Body mass index.
The statistical model additionally includes age, gender, season and medication.

Supplementary table 3. The associations between weight changes and speed of processing among physically active older adults (< 3. hours per week, N = 1702)**

	β	95% CI		P- value
[1]Weight stable	0,110	0,053	0,168	0,000
Weight gain	0,104	0,030	0,177	0,006
Weight loss*				
[2]Weight stable	0,108	0,051	0,166	0,000
Weight gain	0,100	0,026	0,174	0,008
Weight loss*				

β= βeta coefficient. *Compared to weight loss. [1] Model 1: adjusted for age, gender, and baseline cognitive function. [2] Model 2: additional education, smoking and alcohol consumption. **Based on univariate GLM

Supplementary table 4. The associations between weight changes and memory function among physically active adults (< 3. hours per week, N = 1702)**

	β	95% CI		P- value
[1]Weight stable	0,128	0,056	0,201	0,001
Weight gain	0,188	0,095	0,281	0,000
Weight loss*				
[2]Weight stable	0,132	0,059	0,204	0,000
Weight gain	0,198	0,104	0,291	0,000
Weight loss*				

β= βeta coefficient. *Compared to weight loss. [1] Model 1: adjusted for age, gender, and baseline cognitive function. [2] Model 2: additional education, smoking and alcohol consumption. **Based on univariate GLM

Supplementary table 5. The associations between weight changes and executive function among physically active adults

(< 3. hours per week, N = 1702)**

	β	95% CI		P- value
[1]Weight stable	-0,009	-0,077	0,059	0,787
Weight gain	0,002	-0,085	0,089	0,959
Weight loss*				
[2]Weight stable	-0,008	-0,077	0,060	0,808
Weight gain	0,012	-0,075	0,100	0,786
Weight loss*				

β= βeta coefficient. *Compared to weight loss. [1] Model 1: adjusted for age, gender, and baseline cognitive function. [2] Model 2: additional education, smoking and alcohol consumption. **Based on univariate GLM